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**ANNEXURE-II**

**Discipline - wise ARS/NET Syllabus**

## 01. AGRICULTURAL BIOTECHNOLOGY

**Unit 1: Cell Structure and Function**
Prokaryotic and eukaryotic cell architecture, Cell wall, plasma membrane, Structure and function of cell organelles: vacuoles, mitochondria, plastids, golgi apparatus, ER, peroxisomes, glyoxisomes. Cell division, regulation of cell cycle, Protein secretion and targeting, Cell division, growth and differentiation.

**Unit 2: Biomolecules and Metabolism**
Structure and function of carbohydrates, lipids, proteins and nucleic acids, Synthesis of carbohydrate, glycolysis, HMP, Citric acid cycle and metabolic regulation, Oxidative phosphorylation and substrate level phosphorylation, Vitamins, plant hormones. Functional molecules, antioxidants, nutrient precursor, HSPs, anti-viral compounds.

**Unit 3: Enzymology**
Enzymes, structure conformation, classification, assay, isolation, purification andcharacterization, catalytic specificity, mechanism of action, active site, regulation of enzyme activity.

**Unit 4: Molecular Genetics**
Concept of gene, Prokaryotes as genetic system, Prokaryotic and eukaryotic chromosomes, methods of gene isolation and identification, Split genes, overlapping genes and pseudogenes, Organization of prokaryotic and eukaryotic genes and genomes including operan, exon, intron, enhancer promoter sequences and other regulatory elements. Mutation spontaneous, induced and site-directed, recombination in bacteria, fungi and viruses, transformation, transduction, conjugation, transposable elements and transposition.

**Unit 5: Gene Expression**
Expression of genetic information, operon concept, Transcription -mechanism of transcriptionin prokaryotes and eukaryotes, transcription unit, regulatory sequences and enhancers, activators, repressors, co-activators, Co-repressors in prokaryotes and eukaryotes, inducible genes and promoters, Transcription factors post transcriptional modification and protein transport, DNA-protein interaction, Genetic code. Mechanism of translation and its control, post translational modifications.

**Unit 6: Molecular Biology Techniques**
Isolation and purification of nucleic acids. Nucleic acids hybridization: Southern, northernand western blotting hybridization. Immune response monoclonal and polyclonal antibodies and ELISA, DNA sequencing. Construction and screening of genomic and c-DNA libraries. Gel electrophoretic techniques. Polymerase chain reaction, RT-PCR, QRT-PCR spectroscopy, ultracentrifugation, chromatography, FISH, RIA etc.

**Unit 7: Gene Cloning**
Restriction enzymes and their uses. Salient features and uses of most commonly used vectors i.e. plasmids, bacteriophages, phagmids, cosmids, BACs, PACs and YACs, binary vectors, expression vectors. Gene cloning and sub-cloning strategies, chromosome walking, genetic transformation, Risk assessment and IPR.

**Unit 8: Molecular Biology**
Ribosome structure and function. Protein biosynthesis in prokaryotes and ekaryotes. Post-translational modification. Gene regulation, RNA processing and Post-transcriptionalmodifications. Bioprospecting, biofortification, gene pyrimiding and gene fusion, ribozyme technology.

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**Unit 9 : Plant Molecular Biology**
Photoregulation and phytochrome regulation of nuclear and chloroplastic gene expression. photosynthesis, C3& C4 cycle and photorespiration; Molecular mechanism of nitrogen fixation. Molecular biology of various stresses, *viz.* abiotic stresses like drought, salt, heavy metals and temperature; and biotic stresses like bacterial, fungal and viral diseases. Signal transduction and its molecular basis, molecular mechanism of plant hormone action, mitochondrial control of fertility, structure, organization and regulation of nuclear gene concerning storage proteins and starch synthesis.

**Unit 10: Tissue Culture**
Basic techniques in cell culture and somatic cell genetics. Clonal propagation, Concept of cellular totipotency. Another culture, somaclonal and gametoclonal variations. Hybrid embryoculture and embryo rescue, somatic hybridization and cybridization. Application of tissue culture in crop improvement. Secondary metabolite production. *In vitro,* mutagenesis, cryopreservation and plant tissue culture repository.

**Unit 11: Plant Genetic Engineering**
Isolation of genes of economic importance. Gene constructs for tissue-specific expression. Different methods of gene transfer to plants, *viz.* direct and vector mediated. Molecular analysis of transformants. Potential applications of plant genetic engineering for crop improvement, i.e. insect-pest resistance (insect, viral, fungal and bacterial disease resistance), abiotic stress resistance, herbicide resistance, storage protein quality, increasing shelf-life, oil quality, Current status of transgenics, bio-safety norms and controlled field trials and releaseof transgenics (GMOs).

**Unit 12: Molecular Markers and Genomics**
DNA molecular markers: Principles, type and applications; restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA sequences (RAPD), Simple sequence repeats (SSR), Single nucleotide polymorphism (SNP), Structural and functional genomics, gene mapping, genome mapping, gene tagging, transcriptomics, ionomics, metabolomics and proteomics, comparative genomics and application of genomics.



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# CHAPTER 01
# Cell Structure and Function

2015

**1.** Which of the following features fundamentally distinguishes eukaryotic organellar ribosomes from prokaryotic ribosomes?
(A) The sedimentation coefficient and distinct rRNA species
(B) The presence of a 50S large subunit in eukaryotes
(C) A higher sensitivity to cycloheximide in prokaryotes
(D) The complete absence of protein components in organellar ribosomes

2004

**2.** The primary plant cell wall is typically characterized by a high concentration of which structural component?
(A) Lignin networks
(B) Suberin lamellae
(C) Cellulose microfibrils embedded in hemicellulose
(D) Secondary sphingolipid deposits

2008

**3.** What role do viral movement proteins play in the context of plasmodesmata function?
(A) They actively increase the size exclusion limit to facilitate macromolecular transport
(B) They hydrolyze callose to permanently dissolve the middle lamella
(C) They synthesize viral RNA within the symplastic domain
(D) They close the plasmodesmatal pore to prevent host immune responses

2022

**4.** According to the fluid mosaic model, which lipid class is most directly responsible for the formation of membrane microdomains or "lipid rafts" in plant plasma membranes?
(A) Phosphatidylserine and cardiolipin
(B) Free fatty acids and triacylglycerols
(C) Diacylglycerol and phosphatidic acid
(D) Sterols and sphingolipids

2022

**5.** Which mechanism primarily drives secondary active transport across the plant plasma membrane?
(A) Direct hydrolysis of ATP by carrier proteins
(B) The electrochemical gradient established by $H^+$-ATPases
(C) The passive diffusion of water through aquaporins
(D) Spontaneous flip-flop of phospholipids

2004

**6.** In the ultra-structure of plant mitochondria, where is the ATP synthase complex primarily localized?
(A) Embedded in the outer mitochondrial membrane
(B) Freely floating in the mitochondrial matrix
(C) Localized on the inner mitochondrial membrane
(D) Anchored to the mitochondrial nucleoid

2017

**7.** The interconversion of plastids allows proplastids in dark-grown seedlings to develop into which specialized organelle prior to light exposure?
(A) Chromoplasts
(B) Amyloplasts
(C) Leucoplasts
(D) Etioplasts

2016

**8.** The unfolded protein response (UPR) is primarily initiated in which cellular compartment when misfolded proteins accumulate?
(A) The rough endoplasmic reticulum
(B) The trans-Golgi network
(C) The smooth endoplasmic reticulum
(D) The primary plant vacuole

2010

**9.** Vesicular trafficking from the rough endoplasmic reticulum to the cis-Golgi network is predominantly mediated by which coat protein complex?
(A) COP-I
(B) COP-II
(C) Clathrin

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(D) Retromer

2019

**10.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The central vacuole plays a crucial role in plant cell expansion.
Reason (R): Tonoplast transporters accumulate solutes in the vacuole, driving osmotic water uptake and generating turgor pressure.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**11.** Which key marker enzyme is specifically utilized to identify peroxisomes in plant cells?
(A) Catalase
(B) Cytochrome c oxidase
(C) RuBisCO
(D) Malate dehydrogenase

2003

**12.** During the germination of oilseeds, the glyoxylate cycle functions to bypass the decarboxylation steps of the TCA cycle. Which of the following pairs of enzymes is unique to the glyoxysome?
(A) Isocitrate dehydrogenase and malate synthase
(B) Citrate synthase and succinate dehydrogenase
(C) Isocitrate lyase and malate synthase
(D) Isocitrate lyase and aconitase

2004

**13.** Cytoplasmic streaming in plant cells is primarily dependent upon the dynamic interaction between which cytoskeletal component and motor proteins?
(A) Microtubules and kinesins
(B) Intermediate filaments and dyneins
(C) Microtubules and myosins
(D) Microfilaments and myosins

2011

**14.** Which phase of the eukaryotic cell cycle is critically regulated by the DNA damage checkpoint to prevent the replication of broken DNA strands?
(A) S phase
(B) G1/S transition
(C) G2/M transition
(D) M phase

2005

**15.** The Anaphase-Promoting Complex (APC/C) drives cell cycle progression primarily through which biochemical activity?
(A) Phosphorylation of specific target protein substrates
(B) Ubiquitinating cyclins and securin for degradation
(C) Acetylation of the n-terminal histone protein tails
(D) Direct proteolytic cleavage of sister cohesin rings

2021

**16.** During mitosis, the spindle assembly checkpoint (SAC) remains active as long as:
(A) The nuclear envelope is fully intact
(B) Sister chromatids have completed homologous recombination
(C) DNA replication in the S phase is incomplete
(D) Any kinetochore remains unattached to spindle microtubules

2006

**17.** The transition from a mature cell plate to a functional primary cell wall during plant cytokinesis involves the extensive replacement of which polysaccharide?
(A) Callose with cellulose
(B) Lignin with cellulose
(C) Pectin with suberin
(D) Hemicellulose with callose

2023

**18.** At which specific stage of prophase I during meiosis does the synaptonemal complex achieve full assembly, aligning homologous chromosomes continuously?
(A) Leptotene
(B) Zygotene
(C) Pachytene
(D) Diplotene

2001

**19.** The genetic consequence of independent assortment occurs primarily due to the random alignment of bivalents during which meiotic phase?
(A) Prophase I
(B) Metaphase I
(C) Anaphase II
(D) Telophase II

2009

**20.** Match the protein targeting components in List I with their corresponding functions in List II.

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List I: (Protein Targeting Components)
P. SRP
Q. Sec61
R. NLS
S. Ran-GTPase
List II: (Functions)
I. Cytoplasm-to-nucleus transport cycle regulation
II. Nuclear import tagging
III. ER translocon channel
IV. Ribosome targeting to ER
(A) P-IV, Q-III, R-II, S-I
(B) P-IV, Q-II, R-III, S-I
(C) P-III, Q-IV, R-I, S-II
(D) P-I, Q-III, R-IV, S-II

2008

**21.** The transport of nuclear-encoded preproteins into the chloroplast stroma strictly requires which translocon complex sequence?
(A) TOM followed by TIM
(B) TOC followed by TIC
(C) SEC followed by TAT
(D) SRP followed by Sec61

2014

**22.** Which molecule drives the directionality of nuclear-cytoplasmic transport by dissociating importin-cargo complexes within the nucleus?
(A) Ran-GDP
(B) ATP synthase
(C) Ran-GTP
(D) Exportin-1

2021

**23.** Cellular totipotency in plant tissue culture is defined as the ability of a single differentiated cell to:
(A) Undergo apoptosis upon sensing high levels of stress
(B) Expand its central vacuole to achieve maximum turgor
(C) Fuse with a neighboring cell to form a single syncytium
(D) Regenerate into a complete and fertile adult plant

2011

**24.** Vacuolar-type programmed cell death (PCD) in plants is distinctively characterized by:
(A) Tonoplast rupture and release of hydrolytic enzymes
(B) Rapid swelling and bursting of mitochondrial membranes
(C) Plasma membrane rupture with cytosolic content leakage
(D) Vacuolar shrinkage halting essential cellular metabolism

2012

**25.** Which statement correctly differentiates eukaryotic from prokaryotic genome organization?
(A) Eukaryotic genomes lack histone proteins
(B) Eukaryotic DNA is linear and enclosed by a nuclear envelope
(C) Prokaryotic genomes are extensively compartmentalized within a double membrane
(D) Prokaryotes possess multiple linear chromosomes with telomeres

2003

**26.** Which layer of the plant cell wall is synthesized first and is primarily composed of pectin?
(A) Primary wall
(B) Secondary wall
(C) Middle lamella
(D) Tertiary wall

2023

**27.** Identify the INCORRECT pair regarding plasmodesmata.
(A) Symplastic transport - Movement through the cytosol
(B) Size exclusion limit - Regulates the mass of molecules
(C) Primary plasmodesmata formed post-cytokinesis across walls
(D) Desmotubule - A constricted strand of endoplasmic reticulum

2009

**28.** Which of the following is most relevant to maintaining plant plasma membrane fluidity during low-temperature acclimation?
(A) Increasing the proportion of cis-unsaturated fatty acids
(B) Decreasing the proportion of sterols
(C) Increasing the saturation level of phospholipid fatty acyl chains
(D) Cross-linking of integral membrane proteins

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2015

**29.** Facilitated diffusion differs from active transport primarily because it:
(A) Directly requires ATP hydrolysis
(B) Moves molecules against gradients
(C) Utilizes non-specific lipid channels
(D) Relies on existing electrochemical gradients

2010

**30.** Consider the following statements about the plant mitochondrial genome:
I. It is significantly larger and more variable in size than mammalian mitochondrial genomes.
II. It exists as a single, invariable circular molecule in all plant tissues.
III. It contains genes encoding some components of the electron transport chain.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) I
(D) II

2001

**31.** The primary function of amyloplasts in non-photosynthetic plant tissues is the:
(A) Storage of synthesized starch
(B) Synthesis of carotenoids
(C) Production of ATP via cyclic electron flow
(D) Accumulation of essential oils

2011

**32.** Which cellular process relies heavily on N-linked glycosylation within the rough endoplasmic reticulum?
(A) Synthesis of structural cellulose microfibrils
(B) Correct folding of newly synthesized glycoproteins
(C) Targeted degradation of ubiquitin-tagged proteins
(D) Enzymatic hydrolysis of stored neutral lipids

2017

**33.** The cis-Golgi network is primarily responsible for:
(A) Receiving COP-II coated vesicles from the ER
(B) Packaging secretory proteins into COP-II vesicles
(C) Synthesizing host-defense callose deposits
(D) Direct fusion with the plasma membrane

2008

**34.** Which class of organic compounds is typically sequestered in the plant vacuole to deter herbivory?
(A) Primary metabolic enzymes
(B) Ribosomal RNA subunits
(C) Membrane phospholipids
(D) Secondary alkaloids and tannins

2005

**35.** During the C2 photorespiratory cycle, peroxisomes coordinate directly with which two other organelles?
(A) Chloroplasts and the Golgi apparatus
(B) Mitochondria and the endoplasmic reticulum
(C) The nucleus and the central vacuole
(D) Chloroplasts and mitochondria

2016

**36.** Which process represents the critical metabolic role of the glyoxylate cycle in germinating seeds?
(A) Net conversion of fatty acids into carbohydrates
(B) Conversion of stored carbohydrates to amino acids
(C) Synthesis of triacylglycerols from sucrose
(D) Photophosphorylation of ADP

2012

**37.** Cortical microtubules in plant cells directly govern the directional deposition of which extracellular component?
(A) Cellulose microfibrils in the cell wall
(B) Pectin in the middle lamella
(C) Lignin in the secondary cell wall
(D) Suberin in the Casparian strip

2009

**38.** The restriction point in the eukaryotic cell cycle represents a critical decision-making phase occurring during:
(A) The G2 phase
(B) Late S phase
(C) Late G1 phase
(D) Prophase

2003

**39.** Which statement INCORRECTLY describes the regulation of Cyclin-Dependent Kinases (CDKs)?
(A) They are constitutively active throughout the cell cycle
(B) Their activation requires binding to stage-specific cyclins
(C) They can be inhibited by CDK inhibitors (CKIs)
(D) Their activity dictates the progression through cell cycle checkpoints

2024

**40.** Consider the following statements about mitosis:
I. Sister chromatid cohesion is established during S phase.
II. Kinetochores mediate attachment to spindle microtubules.

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III. Chromosome condensation peaks during telophase.
IV. The nuclear envelope reforms during prophase.

Which of the above statement(s) is/are correct?
(A) I and II
(B) II, III and IV
(C) I, II and III
(D) II and III

2015

**41.** The initial structural framework of the plant cell plate is formed by the fusion of vesicles derived from the:
(A) Mitochondria
(B) Endoplasmic reticulum
(C) Peroxisomes
(D) Golgi apparatus

2011

**42.** During which sub-stage of Prophase I does the process of crossing over strictly occur?
(A) Diplotene
(B) Leptotene
(C) Pachytene
(D) Diakinesis

2013

**43.** A defining feature of Meiosis II compared to Meiosis I is the:
(A) Separation of sister chromatids
(B) Separation of homologous chromosomes
(C) Duplication of DNA prior to prophase II
(D) Extensive homologous recombination

2002

**44.** Co-translational translocation into the endoplasmic reticulum is initiated when the signal recognition particle (SRP) binds to a:
(A) Hydrophobic signal peptide on the nascent chain
(B) Stop-transfer sequence on a fully folded protein
(C) Basic nuclear localization signal within the core
(D) Specific mannose-6-phosphate recognition tag

2005

**45.** In the mitochondrial protein import pathway, the TIM complex primarily facilitates translocation across the:
(A) Outer mitochondrial membrane
(B) Thylakoid membrane
(C) Tonoplast
(D) Inner mitochondrial membrane

2018

**46.** The passage of macromolecules through the Nuclear Pore Complex (NPC) requires specific transporter proteins known as:
(A) Importins and exportins
(B) Porins
(C) Sec proteins
(D) Aquaporins

2011

**47.** Asymmetric cell division is a fundamental developmental process in plants primarily because it:
(A) Produces daughter cells with different fates
(B) Prevents mutations during DNA replication
(C) Ensures equal segregation of chloroplasts
(D) Halves the chromosome number

2004

**48.** During developmental leaf senescence, programmed cell death relies heavily on the controlled salvage of nutrients. Which organelle serves as the primary degradation compartment?
(A) The central vacuole
(B) The Golgi apparatus
(C) The smooth endoplasmic reticulum
(D) The nucleus

2021

**49.** Which of the following is NOT a structural feature of a typical prokaryotic nucleoid?
(A) Lack of a nuclear envelope
(B) Abundant histone-based nucleosomes
(C) Covalently closed circular DNA
(D) Direct contact with the cytoplasm

2008

**50.** Consider the following statements about the plant cell wall:
I. Lignin provides mechanical strength and waterproofing.
II. Hemicellulose cross-links cellulose microfibrils.
III. Primary walls consist mainly of suberin.
IV. The middle lamella is rich in pectins.
V. Secondary walls are deposited outside the primary wall.

Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II, IV and V
(C) I, III and V
(D) II, IV and V

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2025

**51.** Secondary plasmodesmata are unique compared to primary plasmodesmata because they are:

(A) Created de novo across existing cell walls
(B) Created exclusively during cell plate formation
(C) Completely devoid of a desmotubule
(D) Only found in non-living xylem vessels

2008

**52.** Which membrane component restricts the uncontrolled diffusion of polar molecules across the plasma membrane?
(A) Integral channel proteins
(B) Peripheral glycoproteins
(C) Membrane cytoskeletal elements
(D) Hydrophobic phospholipid core

2017

**53.** The transport of water across the plant plasma membrane is vastly accelerated by which specific family of integral membrane proteins?
(A) $H^+$-ATPases
(B) Symporters
(C) ABC transporters
(D) Aquaporins

2014

**54.** The extensive folding of the inner mitochondrial membrane into cristae serves primarily to:
(A) House the genetic material of the mitochondrion
(B) Increase surface area for ETC and ATP synthase
(C) Prevent the escape of protons into the cytoplasm
(D) Store calcium ions for signal transduction

2007

**55.** The structural unit of the thylakoid membrane system where light-dependent photosynthetic reactions occur is found exclusively in:
(A) Etioplasts
(B) Chloroplasts
(C) Leucoplasts
(D) Amyloplasts

2017

**56.** What is the primary function of the smooth endoplasmic reticulum in plant cells?
(A) Lipid synthesis and calcium sequestration
(B) Targeted cytosolic ribosome assembly
(C) Proteasomal protein degradation
(D) Genomic mRNA transcription

2017

**57.** Which type of glycosylation is distinctively finalized within the Golgi apparatus?
(A) O-linked glycosylation
(B) N-linked glycosylation
(C) C-linked glycosylation
(D) S-linked glycosylation

2001

**58.** The membrane defining the boundary of the plant central vacuole is termed the:
(A) Plasmalemma
(B) Tonoplast
(C) Symplast
(D) Apoplast

2005

**59.** The biogenesis of plant peroxisomes involves membrane budding directly from which organelle?
(A) The Golgi apparatus
(B) The endoplasmic reticulum
(C) The plasma membrane
(D) The mitochondrion

2024

**60.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Glyoxysomes are essential for the survival of germinating seedlings of oil-bearing plants.
Reason (R): Glyoxysomes possess the enzymes to convert stored lipids into carbohydrates, providing energy before photosynthesis begins.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**61.** Intermediate filaments in eukaryotic cells are fundamentally responsible for:
(A) Mechanical strength and nuclear architecture
(B) Serving as tracks for myosin motors
(C) Forming the mitotic spindle
(D) Catalyzing cellulose synthesis

2017

**62.** If a plant cell fails to pass the G2/M DNA damage checkpoint, which process is immediately halted?
(A) DNA replication
(B) Spindle assembly and mitotic entry
(C) Ribosome synthesis
(D) Cytokinesis

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2022

**63.** CDK inhibitors (CKIs) regulate the cell cycle primarily by:
(A) Enhancing the binding between cyclins and CDKs
(B) Binding complexes to prevent kinase activity
(C) Phosphorylating the APC/C complex
(D) Degrading cyclins via the proteasome

2012

**64.** The primary function of cohesin ring complexes during mitosis is to:
(A) Attach microtubules to the centrosome
(B) Hold sister chromatids together until anaphase
(C) Condense chromatin into distinct chromosomes
(D) Sever the cell plate during cytokinesis

2017

**65.** The phragmoplast, essential for plant cytokinesis, is predominantly constructed from which cytoskeletal elements?
(A) Actin microfilaments and intermediate filaments
(B) Microtubules and actin microfilaments
(C) Septins and keratins
(D) Nuclear lamins

2025

**66.** The visible manifestation of homologous recombination, observable as X-shaped regions during diplotene, is called:
(A) Synapsis
(B) Centromeres
(C) Chiasmata
(D) Kinetochores

2010

**67.** The process of independent assortment in meiosis is best described as the:
(A) Random segregation of homologous chromosomes
(B) Specific crossing over of non-sister chromatids
(C) Replication of DNA between meiosis I and meiosis II
(D) Separation of sister chromatids at anaphase I

2011

**68.** What sequence of events defines the classical signal hypothesis for secreted proteins?
(A) Translation completes in cytoplasm, SRP binds, transport to Golgi
(B) SRP-nascent peptide binding and ER targeting
(C) NLS directs protein to nucleus, exportin mediates ER transfer
(D) Protein enters vacuole directly from ribosomes

2018

**69.** The TOC and TIC translocons interact to facilitate the import of preproteins into which specific chloroplast sub-compartment?
(A) Stroma
(B) Thylakoid lumen
(C) Intermembrane space
(D) Granum

2014

**70.** Match the cellular junctions or transport structures in List I with their defining features in List II.
List I: (Cellular Junctions or Transport Structures)
P. Plasmodesmata
Q. Nuclear Pore Complex
R. Aquaporins
S. Sec61
List II: (Defining Features)
I. Mediates rapid water transport
II. Symplastic cell-to-cell communication
III. Co-translational protein translocation
IV. Importin-mediated cargo transport
(A) P-II, Q-IV, R-I, S-III
(B) P-II, Q-III, R-I, S-IV
(C) P-I, Q-IV, R-II, S-III
(D) P-III, Q-I, R-IV, S-II

2006

**71.** The export of mRNA and ribosomal subunits from the nucleus is directly dependent upon which recognition signal?
(A) NES (Nuclear Export Signal)
(B) NLS (Nuclear Localization Signal)
(C) SRP (Signal Recognition Particle)
(D) Transit peptide

2022

**72.** The induction of cellular differentiation from a totipotent state typically involves the precise orchestration of:
(A) Random genetic mutations
(B) Phytohormone-driven reprogramming
(C) Programmed necrosis
(D) Uncontrolled symmetric cell divisions

2004

**73.** Necrosis-type cell death in plants differs from vacuolar-type PCD primarily in that necrosis is:
(A) Uncontrolled cell rupture from acute stress

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(B) A highly regulated, energy-dependent developmental process
(C) Characterized by chromatin condensation and DNA laddering
(D) Executed by gradual tonoplast permeabilization

2004

**74.** Which process best demonstrates the concept of endosymbiotic theory regarding organelle evolution?
(A) Circular genomes and 70S organellar ribosomes
(B) Cellulose synthesis via plasma membrane rosettes
(C) Vesicular transport between the ER and Golgi
(D) Formation of secondary plasmodesmatal channels

2019

**75.** Lignin deposition in the secondary cell wall primarily confers which physiological advantage to vascular plants?
(A) Hydrophobicity and high compressive strength
(B) Enhanced flexibility for wind resistance
(C) Increased permeability for nutrient diffusion
(D) Attraction of symbiotic fungi

2004

**76.** The symplastic domain of a plant tissue is defined as the:
(A) Continuous network of cell walls and intercellular spaces
(B) Cytoplasmic continuum linked by plasmodesmata
(C) Internal matrix of the central vacuole
(D) Lumen of the endoplasmic reticulum

2010

**77.** Which lipid modification is a hallmark of lipid rafts in the plasma membrane, facilitating receptor clustering?
(A) Enrichment in saturated sphingolipids and sterols
(B) Depletion of all integral proteins
(C) Exclusive presence of cardiolipin
(D) High concentration of unsaturated phosphatidic acid

2021

**78.** Primary active transport differs structurally from passive channels because primary active transporters must possess:
(A) A purely hydrophobic pore
(B) A desmotubule core
(C) ATP-binding and hydrolysis domains
(D) A callose-binding motif

2008

**79.** The outer mitochondrial membrane is generally more permeable to small metabolites than the inner membrane due to the abundant presence of:
(A) ATP synthase
(B) Cytochrome c
(C) Porins (VDACs)
(D) Cardolipins

2011

**80.** Consider the following statements about plastids:
I. Etioplasts develop strictly in the presence of intense light.
II. Chromoplasts synthesize and store carotenoid pigments.
III. Leucoplasts are unpigmented plastids involved in macromolecule synthesis.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II and III
(C) I
(D) III

2001

**81.** In the context of N-linked glycosylation, to which specific amino acid residue is the oligosaccharide precursor attached in the rough ER?
(A) Asparagine
(B) Serine
(C) Threonine
(D) Tyrosine

2019

**82.** Clathrin-coated vesicles are predominantly involved in transport between which two cellular compartments?
(A) ER to cis-Golgi
(B) Chloroplast stroma to thylakoid lumen
(C) TGN to the plasma membrane/endosomes
(D) Nucleus to the rough ER

2025

**83.** The accumulation of organic acids, such as malate and citrate, in plant vacuoles serves primarily to:
(A) Generate ATP directly via substrate-level phosphorylation
(B) Construct the secondary cell wall
(C) Trigger programmed necrosis
(D) Regulate pH and maintain cellular turgor

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2012

**84.** Catalase functions within the peroxisome to mitigate oxidative stress by directly catalyzing the breakdown of:
(A) Superoxide radicals into oxygen and water
(B) Hydrogen peroxide into water and oxygen
(C) Glyoxylate into malate
(D) Uric acid into allantoin

2021

**85.** The enzyme isocitrate lyase cleaves isocitrate into which two distinct molecules during the glyoxylate cycle?
(A) Succinate and glyoxylate
(B) Malate and fumarate
(C) Citrate and oxaloacetate
(D) Acetyl-CoA and α-ketoglutarate

2015

**86.** Which structural parameter dictates the rigidity and distinct polarity of an actin microfilament?
(A) The random copolymerization of tubulin α and β subunits
(B) The cross-linking by lignin polymers
(C) Head-to-tail G-actin assembly with ATP
(D) The symmetric binding of keratin dimers

2018

**87.** The irreversible progression from G1 into S phase relies on the hyperphosphorylation of target proteins that regulate the:
(A) Transcription of S-phase specific genes
(B) Assembly of the synaptonemal complex
(C) Degradation of the nuclear envelope
(D) Cleavage of the cell plate

2005

**88.** Cyclin levels fluctuate during the cell cycle because they are subject to:
(A) Targeted ubiquitin-mediated proteolysis
(B) Continuous transport out of the cell
(C) Spontaneous denaturation at the G2/M boundary
(D) Permanent storage in the central vacuole

2004

**89.** Chromosome condensation is driven primarily by the ATP-dependent activity of which specialized protein complex?
(A) Cohesin
(B) Condensin
(C) Securin
(D) Separase

2014

**90.** Consider the following statements about plant cytokinesis:
I. It is directed by the phragmoplast.
II. Vesicles fuse to form the cell plate.
III. Callose is a key transient component.
IV. It proceeds through a cleavage furrow.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I and II

2018

**91.** The resolution of chiasmata and terminalization occurs progressively during which phase of Prophase I?
(A) Leptotene
(B) Zygotene
(C) Pachytene
(D) Diakinesis

2009

**92.** The segregation of homologous chromosomes, rather than sister chromatids, is uniquely characteristic of:
(A) Mitotic anaphase
(B) Meiotic anaphase II
(C) Meiotic anaphase I
(D) Meiotic metaphase II

NODIA

2005

**93.** What critical component determines the fidelity with which a signal sequence is recognized during co-translational targeting?
(A) N-terminal signal peptide hydrophobicity
(B) The presence of a poly-A tail on the mRNA
(C) The degree of glycosylation on the nascent chain
(D) The size of the completed protein

2014

**94.** The TOM complex recognizes mitochondrial preproteins through interactions with which structural feature?
(A) A C-terminal KDEL sequence
(B) A mannose-6-phosphate residue
(C) A central hydrophobic transmembrane domain
(D) N-terminal amphipathic α-helix signals

2019

**95.** The energy driving the active export of cargo through the Nuclear Pore Complex is ultimately derived from:
(A) ATP hydrolysis by importins
(B) A proton gradient across the nuclear envelope
(C) The Ran-GTPase cycle GTP hydrolysis

(D) Electron transport in the nuclear membrane

2004

**96.** Asymmetric cell division is most visibly instrumental in the formation of which specific epidermal plant structure?
(A) Stomatal guard cells
(B) Mesophyll parenchyma
(C) Xylem vessel elements
(D) Pith collenchyma

2005

**97.** The hallmark event defining the execution phase of vacuolar-type programmed cell death in tracheary element development is:
(A) Vacuolar collapse and cytoplasm clearance
(B) The doubling of nuclear DNA content
(C) The synthesis of excessive primary cell wall
(D) The generation of new plasmodesmata

2014

**98.** Which phenomenon establishes the definitive boundary between prokaryotic and eukaryotic cellular organization?
(A) Internal metabolic compartmentalization
(B) The presence of a rigid cell wall
(C) The utilization of ribosomes for protein synthesis
(D) The reliance on ATP for energetic transactions

2018

**99.** The orientation of cellulose microfibril deposition in the primary cell wall is fundamentally directed by the movement of:
(A) Golgi vesicles along microfilaments
(B) CESA complexes along cortical microtubules
(C) Actin filaments sliding over myosin motors
(D) Endoplasmic reticulum strands through plasmodesmata

2017

**100.** Consider the following statements about plant membrane transport:
I. Primary active transport requires ATP.
II. Secondary active transport depends on a generated ion gradient.
III. Channel proteins typically allow faster transport rates than carrier proteins.
IV. Aquaporins mediate the active transport of water against its gradient.
V. Facilitated diffusion requires specific integral membrane proteins.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) I, III, IV and V
(D) II, III, IV and V

2011

**101.** The size exclusion limit of a typical, unmodified primary plasmodesmata is generally considered to be in the range of:
(A) 1-5 kDa
(B) 50-100 kDa
(C) 100-200 kDa
(D) $>$ 500 kDa

2003

**102.** The concept of membrane fluidity implies that phospholipids and membrane proteins are capable of:
(A) Rapid covalent bonding to form solid matrices
(B) Spontaneous transition into aqueous solutions
(C) Lateral diffusion within the plane of the lipid bilayer
(D) Frequent and rapid transverse "flip-flop" without enzymatic assistance

2011

**103.** Carrier-mediated facilitated diffusion exhibits saturation kinetics. This indicates that:
(A) The transport rate increases indefinitely with concentration
(B) Transport is limited by the number of available carrier proteins
(C) The transport requires continuous ATP hydrolysis
(D) Carrier proteins transport molecules strictly against their gradient

NODIA

2025

**104.** Which structural adaptation of the inner mitochondrial membrane accommodates the vast assembly of respiratory complexes?
(A) Formation of the smooth outer contour
(B) Absence of lipid components
(C) Invaginations known as cristae
(D) Pores formed by VDACs

2006

**105.** Chloroplast development from proplastids in the presence of light involves the massive biosynthesis of which critical structural component?
(A) The prolamellar body
(B) The thylakoid membrane system
(C) Secondary cell wall lignin
(D) Extracellular callose

2017

**106.** The Unfolded Protein Response (UPR) restores ER homeostasis primarily by:
(A) Upregulating ER-resident chaperones
(B) Increasing the synthesis of all structural proteins
(C) Inducing immediate plasma membrane rupture
(D) Shutting down the synthesis of membrane phospholipids

2022

**107.** Which distinct modification differentiates trans-Golgi network functions from those of the cis-Golgi?
(A) The initial reception of rough ER vesicles
(B) The synthetic signal recognition particles
(C) The storage of calcium ions
(D) Protein sorting into clathrin-coated vesicles

2016

**108.** Tonoplast intrinsic proteins (TIPs) are a specialized class of aquaporins essential for:
(A) Facilitating the exchange of ADP and ATP across the mitochondrial membrane
(B) Regulating osmotic balance and rapid water flux across the vacuolar membrane
(C) Pumping protons into the extracellular space
(D) Anchoring microtubules to the plasma membrane

2008

**109.** The C2 photorespiratory cycle effectively recovers carbon lost during oxygenation by RuBisCO, but requires the close coordination of the chloroplast, peroxisome, and mitochondrion to convert:
(A) Glycolate to glycerate
(B) Acetyl-CoA to succinate
(C) Ribulose-1,5-bisphosphate to glucose
(D) Malate to pyruvate

2025

**110.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The glyoxylate cycle is completely absent in mature, photosynthetically active leaves.
Reason (R): The enzymes isocitrate lyase and malate synthase are strictly localized to glyoxysomes, which rapidly degrade or transition into peroxisomes once photosynthesis is established.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**111.** During nuclear division, the proper positioning of the mitotic spindle in a plant cell relies heavily on the pre-mitotic formation of the:
(A) Preprophase band of microtubules
(B) Casparian strip
(C) Primary cell wall
(D) Central vacuole

2010

**112.** The G1/S transition is frequently termed a "restriction point" because bypassing it commits the cell to:
(A) Apoptosis
(B) Immediate cytokinesis
(C) A complete round of DNA replication
(D) Entering a quiescent G0 state

2022

**113.** If a loss-of-function mutation occurs in an Anaphase-Promoting Complex (APC/C) gene, the cell will most likely:
(A) Rapidly transition from S to G2 phase
(B) Undergo premature cytokinesis
(C) Condense its chromatin excessively during interphase
(D) Arrest in metaphase from failed securin loss

2005

**114.** Proper kinetochore attachment during metaphase generates tension across the sister chromatids, a physical state that directly:
(A) Initiates DNA replication
(B) Silences the Spindle Assembly Checkpoint
(C) Activates cyclin synthesis
(D) Triggers nuclear envelope breakdown

2022

**115.** Callose synthases are heavily targeted to the cell plate during early cytokinesis to:
(A) Form a transient matrix before cellulose entry
(B) Induce immediate programmed cell death
(C) Lignify the newly formed cell wall
(D) Synthesize the middle lamella pectins

2012

**116.** The synaptonemal complex is distinctively composed of a tripartite proteinaceous structure primarily meant to:
(A) Facilitating recombination by aligning

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homologs
(B) Segregate sister chromatids
(C) Prevent crossing over events
(D) Attach chromosomes to the spindle poles

2015

**117.** Non-disjunction occurring during Meiosis I results from the failure of:
(A) Homologous chromosomes to separate
(B) Sister chromatids to separate
(C) The nuclear envelope to breakdown
(D) Spindle fibers to form

2011

**118.** In the Sec61 translocon, the central pore is structurally designed to:
(A) Exclude exclusively positively charged ions
(B) Allow unfolded polypeptide passage through membranes
(C) Directly hydrolyze ATP to pull the protein across
(D) Fold proteins before they enter the ER lumen

2021

**119.** Chloroplast precursor proteins synthesized in the cytosol possess an N-terminal transit peptide that is typically:
(A) Cleaved by processing peptidases upon entry
(B) Retained permanently as an integral membrane anchor
(C) Glycosylated prior to import
(D) Exported back to the cytosol after import

2014

**120.** Match the cell cycle regulators in List I with their primary functions in List II.
List I: (Cell Cycle Regulators)
P. G1 Cyclins
Q. Securin
R. Separase
S. APC/C
List II: (Primary Functions)
I. Inhibits cohesin cleavage until anaphase
II. Cleaves cohesin to allow chromatid separation
III. Targets proteins for ubiquitin-mediated degradation
IV. Activates CDKs to prepare for DNA replication
(A) P-IV, Q-I, R-II, S-III
(B) P-III, Q-I, R-IV, S-II
(C) P-IV, Q-II, R-I, S-III
(D) P-I, Q-IV, R-II, S-III

NODIA

2024

**121.** The import of proteins into the nucleus via importins differs fundamentally from ER translocation because nuclear import:
(A) Demands proteins to be completely unfolded
(B) Accommodates fully folded proteins through pores
(C) Is strictly co-translational
(D) Does not require targeting signals

2022

**122.** Which molecular hallmark is commonly associated with the initiation of senescence-associated programmed cell death in leaves?
(A) Rapid increase in photosynthetic rate
(B) Synthesis of massive starch reserves
(C) Thickening of the secondary cell wall
(D) SAG upregulation and chlorophyll loss

2008

**123.** The execution of developmental programmed cell death in the formation of aerenchyma tissue typically involves:
(A) Ethylene-induced vacuolar rupture
(B) Suppression of hydrolytic enzymes
(C) Increased mitotic index in adjacent cells
(D) The formation of massive secondary vascular tissue

2019

**124.** Eukaryotic DNA is highly organized and compacted. Which fundamental unit of chromatin packaging is strictly absent in prokaryotes?
(A) The nucleosome
(B) The nucleoid
(C) The supercoil
(D) The plasmid

2016

**125.** The deposition of specialized suberin lamellae is most characteristic of which specific plant cell type to control apoplastic transport?
(A) Apical meristematic cells
(B) Root endodermal cells (Casparian strip)
(C) Epidermal trichomes
(D) Phloem sieve tube elements

2004

**126.** A distinctive feature of primary plasmodesmata compared to secondary plasmodesmata is that primary plasmodesmata:
(A) Contain a desmotubule derived from the ER during division
(B) Are exclusively formed by enzymatic degradation of mature cell walls
(C) Allow the unrestricted passage of whole

organelles
(D) Lack any actin or myosin components

2004

**127.** Membrane fluidity is critically balanced. At high temperatures, sterols in the plant plasma membrane act to:
(A) Restrict fatty acid movement to prevent excessive fluidity
(B) Completely solidify the membrane
(C) Increase the proportion of unsaturated bonds
(D) Detach peripheral proteins from the bilayer

2017

**128.** $H^+$-ATPases utilize the energy of ATP hydrolysis to:
(A) Extrude apoplastic protons to generate gradients
(B) Transport protons into the cytosol
(C) Symport sucrose directly
(D) Synthesize ATP from ADP and Pi

2015

**129.** The genome of a plant mitochondrion is highly complex due to a high frequency of:
(A) Telomere shortening
(B) Intramolecular recombination yielding subgenomic circles
(C) RNA editing errors
(D) Complete horizontal gene transfer from chloroplasts

2021

**130.** Consider the following statements about plastid interconversion:
I. Etioplasts convert to chloroplasts upon light perception.
II. Chloroplasts can senesce into gerontoplasts.
III. Amyloplasts permanently lose the ability to differentiate back to proplastids.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I

2014

**131.** In addition to protein synthesis, the endoplasmic reticulum serves as the primary cellular sink for which crucial signaling ion?
(A) Sodium
(B) Potassium
(C) Calcium
(D) Chloride

2005

**132.** The process of O-linked glycosylation, primarily occurring in the Golgi, involves the attachment of sugars to which specific amino acid residues?
(A) Asparagine and Glutamine
(B) Serine and Threonine
(C) Cysteine and Methionine
(D) Proline and Glycine

2001

**133.** The immense volume of a mature plant central vacuole is largely maintained by the continuous osmotic influx of water, which is driven by:
(A) Active transport of water via ATPases
(B) High concentrations of accumulated solutes
(C) Complete exclusion of all ions
(D) Secretion of structural cell wall proteins

2010

**134.** Unlike in animal cells, the β-oxidation of fatty acids in plant cells occurs exclusively within which organelle?
(A) Peroxisomes
(B) Mitochondria
(C) Lysosomes
(D) Endoplasmic reticulum

2006

**135.** The transition from lipid-rich endosperm to photosynthetic capacity in seedlings is marked by the metabolic decline of which specific organelle?
(A) The glyoxysome
(B) The central vacuole
(C) The rough endoplasmic reticulum
(D) The nucleus

2013

**136.** Cortical microtubules function as crucial positional cues that directly guide the membrane trajectories of:
(A) Cellulose synthase (CESA) complexes
(B) Nuclear import complexes
(C) Tonoplast aquaporins
(D) Plasmodesmatal desmotubules

2013

**137.** Which phase of the cell cycle is predominantly responsible for the duplication of the centrosome (or its equivalent microtubule-organizing center in plants)?
(A) G1 phase
(B) S phase
(C) M phase
(D) G0 phase

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2003

**138.** The degradation of Cyclin B is an essential prerequisite for the cell to transition from:
(A) Metaphase to Anaphase
(B) G1 to S phase
(C) S phase to G2 phase
(D) Prophase to Metaphase

2006

**139.** Spindle microtubules attach to chromosomes at specialized multiprotein structures located at the centromere known as:
(A) Centrosomes
(B) Kinetochores
(C) Telomeres
(D) Nucleosomes

2021

**140.** Consider the following statements about plant cytokinesis:
I. It is initiated by a contractile actin ring.
II. It requires phragmoplast expansion towards the cell periphery.
III. It builds the cell plate centrifugally.
IV. It utilizes Golgi-derived vesicles.
Which of the above statement(s) is/are INCORRECT?
(A) I
(B) I and IV
(C) II and III
(D) IV

2012

**141.** The failure of homologous chromosomes to cross over during prophase I usually results in:
(A) Improper segregation and aneuploidy
(B) Immediate cell death prior to metaphase I
(C) Increased stability of the bivalent at the metaphase plate
(D) A secondary round of DNA replication

2016

**142.** The second meiotic division (Meiosis II) is mechanically most similar to:
(A) Binary fission in prokaryotes
(B) Mitosis, but occurring in haploid cells
(C) Meiosis I, but without a spindle
(D) Cytokinesis in plant tissues

2001

**143.** Which molecule acts as the primary receptor for the nascent signal peptide and subsequently docks to the ER membrane?
(A) Signal Recognition Particle (SRP)
(B) Ran-GTP
(C) Signal Peptidase
(D) BiP (Binding Protein)

2019

**144.** The TIC complex (Translocon at the Inner Envelope Membrane of Chloroplasts) specifically facilitates the transport of proteins into the:
(A) Chloroplast intermembrane space
(B) Chloroplast stroma
(C) Cytoplasm
(D) Plant vacuole

2019

**145.** In the Ran-GTPase cycle regulating nuclear transport, the high concentration of Ran-GTP in the nucleus is maintained by:
(A) Chromatin-bound Ran-GEF factors
(B) Ran-GAP (GTPase Activating Protein)
(C) Importin-$\alpha$
(D) Exportin-$\beta$

2009

**146.** A critical hormonal ratio often manipulated to induce cellular totipotency and shoot/root regeneration in tissue culture involves:
(A) Ethylene and Abscisic Acid
(B) Auxin and Cytokinin
(C) Gibberellin and Brassinosteroid
(D) Jasmonic Acid and Salicylic Acid

2022

**147.** During pathogen-triggered hypersensitive response (HR), plant cells rapidly undergo which form of cell death to restrict pathogen spread?
(A) Necrosis and rapid vacuolar rupture
(B) Gradual developmental senescence
(C) Autophagic recycling of chloroplasts
(D) Mitotic catastrophe

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2012

**148.** Which structural property of prokaryotic DNA permits coupled transcription and translation?
(A) The presence of a rigid peptidoglycan wall
(B) The lack of any ribosomal structures
(C) The high proportion of introns
(D) The lack of a nuclear envelope

2006

**149.** The cross-linking of cellulose microfibrils by hemicellulose in the primary cell wall directly confers:
(A) Tensile strength and growth extensibility
(B) Absolute rigidity with no capacity for expansion
(C) Enzymatic degradation capability
(D) Complete impermeability to water

2008

**150.** Consider the following statements about plasmodesmata:
I. They form a symplastic continuum.
II. The desmotubule is a constricted ER strand.
III. Callose deposition at the neck restricts their permeability.
IV. Viral movement proteins decrease their size exclusion limit.
V. They allow diffusion of transcription factors under certain conditions.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2013

**151.** Which biophysical principle governs the spontaneous formation of a lipid bilayer in aqueous environments?
(A) Amphipathic lipids driving hydrophobic tails together
(B) The covalent bonding between adjacent phosphate groups
(C) The active transport of water away from the lipids
(D) The enzymatic cross-linking of fatty acids

2023

**152.** Aquaporins are strictly classified as:
(A) Active transport pumps
(B) Facilitated diffusion channels
(C) Ion-selective carriers
(D) Endocytotic vesicles

2008

**153.** What critical component is encoded by the mitochondrial genome and is essential for the function of the electron transport chain?
(A) Select core subunits of oxidase and dehydrogenase
(B) All subunits of ATP synthase
(C) Most proteins of the Krebs cycle
(D) Ribulose bisphosphate carboxylase

2001

**154.** Amyloplasts can sense gravity in root columella cells by functioning as:
(A) Statoliths sedimenting to the lowest point
(B) Light receptors that orient root growth away from the surface
(C) Hormone synthesizing centers that release auxin directly
(D) Contractile organelles that pull the root downward

2005

**155.** The misfolded proteins targeted by the Unfolded Protein Response (UPR) are ultimately degraded by which cellular machinery?
(A) Cytosolic 26S proteasome via ERAD pathways
(B) The lysosome
(C) The peroxisomal matrix enzymes
(D) The central vacuole proteases

2019

**156.** The retrieval of resident ER proteins from the cis-Golgi is mediated by the binding of a specific C-terminal retention motif to receptors packaged in:
(A) COP-I vesicles
(B) COP-II vesicles
(C) Clathrin-coated vesicles
(D) Secretory vesicles

2019

**157.** Tonoplast antiporters frequently utilize which specific gradient to sequester toxic ions like Na+ into the central vacuole?
(A) Proton ($H^+$) gradient
(B) ATP gradient
(C) Calcium ($Ca^{2+}$) gradient
(D) Sucrose gradient

2018

**158.** The ER-resident chaperone protein BiP (Binding Protein) primarily functions to:
(A) Assist in the folding of newly synthesized polypeptides
(B) Tag proteins for degradation by the proteasome
(C) Phosphorylate cyclin-dependent kinases
(D) Form the structural core of the desmotubule

2025

**159.** The conversion of stored lipids to sucrose during seed germination is crucial because sucrose is:
(A) The primary transport sugar for the growing axis
(B) Highly toxic to soil pathogens
(C) Required directly to synthesize DNA
(D) The structural basis of the middle lamella

2005

**160.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The orientation of newly synthesized cellulose microfibrils generally aligns with the orientation of underlying cortical microtubules.

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Reason (R): Cortical microtubules physically transport cellulose microfibrils across the plasma membrane into the cell wall.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**161.** DNA damage checkpoints ensure genomic integrity. A persistent double-strand break in G2 will directly inhibit the activation of:
(A) M-phase CDK complexes
(B) G1 cyclins
(C) The Anaphase-Promoting Complex
(D) Ribosomal RNA synthesis

2001

**162.** The destruction of Cyclin A and B relies on their tagging with a polyubiquitin chain. This tagging is the direct enzymatic function of:
(A) The proteasome core
(B) The APC/C E3 ubiquitin ligase
(C) Cyclin-Dependent Kinases
(D) Securin

2022

**163.** The spindle assembly checkpoint (SAC) relies on the generation of an inhibitory signal, primarily mediated by the Mad2 protein, originating from:
(A) Unattached kinetochores
(B) Fully attached kinetochores
(C) The nucleolus
(D) The cleavage furrow

2001

**164.** The formation of the plant cell plate is distinctly defined as a process of cytokinesis that is:
(A) Centrifugal, growing outward from the center of the cell
(B) Centripetal, growing inward from the plasma membrane
(C) Random, assembling simultaneously throughout the cytoplasm
(D) Unidirectional, strictly from the apical to basal pole

2009

**165.** During meiosis I, the phenomenon of "homologous chromosome pairing" requires the chromosomes to undergo extensive movements to find their specific partners. This movement is facilitated by their attachment to the:
(A) Plasma membrane via actin
(B) Nuclear envelope via telomeres
(C) Central vacuole via tonoplast strands
(D) Nucleolus via ribosomal RNA

2015

**166.** A defining characteristic of diakinesis is the:
(A) Initiation of the synaptonemal complex
(B) Max bivalent condensation and nucleolus dispersal
(C) Replication of mitochondrial DNA
(D) Separation of sister chromatids

2001

**167.** Following the completion of Meiosis II in an anther, the resulting four haploid cells are generally enclosed in a common:
(A) Callose wall
(B) Cellulosic primary wall
(C) Lignified secondary wall
(D) Pectin sheath

2021

**168.** The signal peptide of a secretory protein is typically cleaved off immediately after translocation into the ER lumen by:
(A) Signal peptidase
(B) The 26S proteasome
(C) Trypsin
(D) BiP chaperone

2015

**169.** Mitochondrial targeting signals (presequences) are highly distinct in their structure, usually forming:
(A) Amphipathic α-helices with positive residues
(B) A negatively charged β-sheet
(C) A highly glycosylated random coil
(D) A strictly hydrophobic transmembrane domain

NODIA

2013

**170.** Match the organelle in List I with its unique imported or targeted marker/signal in List II.
List I: (Organelle)
P. Chloroplast
Q. Nucleus
R. ER
S. Mitochondrion
List II: (Unique Imported or Targeted Marker/ Signal)
I. N-terminal transit peptide
II. NLS (often rich in Arg/Lys)
III. Amphipathic presequence
IV. KDEL retention sequence
(A) P-I, Q-II, R-IV, S-III
(B) P-II, Q-I, R-III, S-IV

(C) P-I, Q-III, R-II, S-IV
(D) P-III, Q-II, R-IV, S-I

2005

**171.** Importin-β interacts directly with the Nuclear Pore Complex by binding transiently to:
(A) Nucleoporin FG-repeats (Phe-Gly)
(B) Phospholipid heads
(C) Cellulose microfibrils
(D) ATP synthase complexes

2001

**172.** Totipotency implies that cellular differentiation is largely:
(A) Epigenetic and hormonal-signal reversible
(B) Irreversible, involving permanent DNA deletion
(C) Driven by irreversible chromatin fragmentation
(D) Restricted entirely to zygotic cells

2009

**173.** During vacuolar-type programmed cell death (e.g., xylem tracheary element differentiation), the final autolysis of the cell contents is triggered specifically by:
(A) Tonoplast collapse and hydrolytic release
(B) The synthesis of new mitochondria
(C) The rapid closure of plasmodesmata
(D) Hyperpolarization of the plasma membrane

2014

**174.** Which process most distinctly highlights the biochemical complexity of eukaryotic membrane compartmentation compared to prokaryotes?
(A) Coat protein-mediated vesicle budding and fusion
(B) ATP synthesis across the plasma membrane
(C) Diffusion of gases across the lipid bilayer
(D) Ribosomal translation of mRNA

2003

**175.** The specific pattern of secondary cell wall thickenings (annular, helical, reticulate) in xylem elements is directly templated by:
(A) The distribution of cortical microtubules
(B) The size of the central vacuole
(C) The positioning of the nucleolus
(D) The number of chloroplasts

2005

**176.** Which structural component specifically traverses the central pore of a primary plasmodesma?
(A) Desmotubule made of tightly appressed ER lipid
(B) An actin microfilament bundle
(C) A continuous strand of the nuclear envelope
(D) A rigid cylinder of crystallized callose

2001

**177.** The asymmetric distribution of specific lipids in the outer and inner leaflets of the plasma membrane is actively maintained by:
(A) Flippases and floppases utilizing ATP
(B) Random diffusion
(C) The continuous physical mechanical stress of the cell wall
(D) The electrochemical gradient alone

2025

**178.** Arrange the following events in the correct sequence for secondary active transport (e.g., a sucrose-$H^+$ symporter):
I. Binding of extracellular sucrose and $H^+$ to the symporter.
II. Primary transport by $H^+$-ATPase establishes an outward $H^+$ gradient.
III. Conformational change of the symporter protein.
IV. Release of sucrose and $H^+$ into the cytoplasm.
Which of the following is the correct sequence?
(A) II, I, III, IV
(B) I, II, III, IV
(C) II, III, I, IV
(D) I, III, IV, II

2007

**179.** Order the following stages of prophase I in their correct developmental arrangement:
I. Pachytene
II. Diplotene
III. Leptotene
IV. Diakinesis
V. Zygotene
Which of the following is the correct sequence?
(A) III, V, I, II, IV
(B) V, III, I, II, IV
(C) III, I, V, II, IV
(D) III, V, II, I, IV

2024

**180.** Which component is strictly localized to the mitochondrial matrix?
(A) TCA cycle enzymes (except succinate dehydrogenase)
(B) Cytochrome c
(C) Porin channels
(D) The $F_0$ subunit of ATP synthase

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2006

**181.** The differentiation of leucoplasts into highly specialized amyloplasts in root columella tissues is essential for:
(A) Photosynthesis in the dark
(B) Gravitropism
(C) Synthesizing floral pigments
(D) Pathogen defense

2015

**182.** A failure in the function of the Signal Recognition Particle (SRP) would most immediately result in:
(A) Secretory protein translation in the cytosol instead of ER
(B) Complete cessation of all cellular transcription
(C) Rapid unfolding of existing ER-resident proteins
(D) Uncontrolled vesicular fusion with the plasma membrane

2021

**183.** The trans-Golgi network (TGN) functions primarily as a major sorting station. Which vesicle destination is generally NOT routed through the TGN?
(A) The inner mitochondrial membrane
(B) The vacuole
(C) The plasma membrane
(D) The extracellular space

2012

**184.** Plant vacuoles contribute significantly to cellular detoxification by sequestering heavy metals and xenobiotics. This process often relies on tagging these toxic compounds with:
(A) Polyubiquitin
(B) Lignin precursors
(C) Mannose-6-phosphate
(D) Glutathione or phytochelatins

2008

**185.** The high concentration of internal oxygen generated during the C2 photorespiratory cycle in peroxisomes is safely managed by:
(A) Catalase-mediated peroxide to water/oxygen conversion
(B) Immediate export to the stroma
(C) Binding completely to hemoglobin-like molecules
(D) Incorporation into structural lipid membranes

2016

**186.** Which metabolic transformation is uniquely facilitated by the glyoxysome during early seedling growth?
(A) Conversion of glucose to fatty acids
(B) Converting lipid-derived acetyl-CoA to 4-carbon acids
(C) Fixation of carbon dioxide into sugars
(D) Degradation of proteins into free amino acids

2004

**187.** Intermediate filaments in plants are generally considered to be:
(A) Distinct from animal keratin and less prominent
(B) Highly abundant and primarily responsible for cellulose synthesis
(C) The main drivers of cytoplasmic streaming
(D) The sole structural component of the phragmoplast

2022

**188.** A cell permanently arrested at the G1 restriction point is typically classified as residing in which state?
(A) G2 phase
(B) G0 phase (quiescence)
(C) Early S phase
(D) Apoptosis

2018

**189.** The catalytic activity of Cyclin-Dependent Kinases (CDKs) specifically transfers a phosphate group from ATP to which amino acid residues on target proteins?
(A) Serine and Threonine
(B) Phenylalanine and Tryptophan
(C) Cysteine and Methionine
(D) Glutamate and Aspartate

2009

**190.** During anaphase of mitosis, the pulling forces that separate sister chromatids towards opposite poles are generated primarily by:
(A) Depolymerizing microtubules and motor activity
(B) The rapid polymerization of actin filaments
(C) The contraction of the nuclear envelope
(D) The swelling of the central vacuole

2016

**191.** The structural transition from callose to cellulose in the developing cell plate is functionally significant because it provides:
(A) Temporary flexibility and later structural rigidity

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(B) Absolute impermeability to water and solutes
(C) A matrix that completely prevents plasmodesmata formation
(D) An immediate trigger for secondary wall lignification

2005

**192.** During zygotene, the initiation of the synaptonemal complex ensures that:
(A) Homologous chromosomes align precisely along their entire lengths
(B) Sister chromatids separate prematurely
(C) DNA replication restarts to repair errors
(D) Crossing over is completely suppressed

2018

**193.** The genetic diversity generated by independent assortment is mathematically dependent on the:
(A) Number of crossing over events per chromosome
(B) Size of the individual chromosomes
(C) Number of meiotic divisions
(D) Number of homologous chromosome pairs (bivalents)

2007

**194.** The structural core of the Sec61 translocon complex is evolutionarily highly conserved, reflecting its fundamental role in:
(A) Passing polypeptide chains across a lipid bilayer
(B) Generating ATP from proton gradients
(C) Facilitating the diffusion of simple sugars
(D) Synthesizing sterols

2017

**195.** To prevent precursor proteins from folding prematurely in the cytosol before mitochondrial import, they are bound by:
(A) Cytosolic chaperones such as Hsp70
(B) The Signal Recognition Particle (SRP)
(C) Clathrin coats
(D) Exportins

2005

**196.** The directionality of nuclear transport is driven by a steep gradient of Ran-GTP. Where is the concentration of Ran-GTP the highest?
(A) Within the nucleoplasm
(B) In the cytosol
(C) In the ER lumen
(D) In the intermembrane space of the nuclear envelope

2012

**197.** The concept of asymmetric cell division is central to plant embryogenesis, specifically defining the initial division of the zygote to form:
(A) A small apical cell and a larger basal cell
(B) Two identical apical cells
(C) A mass of unorganized callus tissue
(D) Directly into four separate microspores

2019

**198.** Programmed cell death (PCD) is distinctively NOT associated with which physiological state?
(A) Xylem vessel element maturation
(B) Hypersensitive response to pathogens
(C) Uncontrolled bursting from acute freezing damage
(D) Developmental leaf senescence

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2016

**199.** While eukaryotic cells utilize internal membrane compartments to localize enzymes, prokaryotic cells generally perform oxidative phosphorylation:
(A) Directly across their plasma membrane
(B) Within primitive mitochondrial precursors
(C) In the nucleoid region
(D) Exclusively within free-floating vesicles

2011

**200.** Pectins in the middle lamella regulate cell adhesion and expansion primarily through their ability to cross-link via which divalent cation?
(A) Calcium ($Ca^{2+}$)
(B) Magnesium ($Mg^{2+}$)
(C) Potassium ($K^{+}$)
(D) Sodium ($Na^{+}$)

2018

**201.** Which structural feature dynamically regulates the permeability of plasmodesmata in response to mechanical stress or pathogen attack?
(A) The synthesis of new cellulose microfibrils
(B) Reversible callose deposition at the neck
(C) The permanent destruction of the desmotubule
(D) The expansion of the central vacuole

2016

**202.** Sphingolipids in the plant plasma membrane differ functionally from typical phospholipids because they are primarily involved in:
(A) Rigid lipid raft formation and signaling
(B) Energy storage
(C) Rapid transverse flip-flop to adapt to heat
(D) Forming the purely hydrophobic core of porins

2022

**203.** Which ion is primarily transported out of the plant cell by the plasma membrane $H^+$-ATPase to establish the resting membrane potential?
(A) $Na^+$
(B) $K^+$
(C) $H^+$
(D) $Ca^{2+}$

2015

**204.** The organization of the mitochondrial genome differs distinctly from the nuclear genome due to its:
(A) Presence in multiple circular and subgenomic copies
(B) Strict linear structure enclosed in nucleosomes
(C) Inheritance exclusively from the paternal line in most angiosperms
(D) Total reliance on RNA rather than DNA

2010

**205.** The conversion of a mature chloroplast into a chromoplast during fruit ripening involves the massive breakdown of the thylakoid membrane and the accumulation of:
(A) Carotenoids and specialized plastoglobuli
(B) Starch grains
(C) Lignin precursors
(D) Water-soluble anthocyanins

2005

**206.** Calcium storage in the endoplasmic reticulum is essential for rapid intracellular signaling. Its release into the cytosol is primarily triggered by:
(A) Opening of ligand-gated IP3 receptor channels
(B) Direct voltage changes across the plasma membrane
(C) Random diffusion through the lipid bilayer
(D) The sudden collapse of the ER membrane

2014

**207.** COP-I coated vesicles are primarily responsible for:
(A) Retrograde transport from Golgi back to ER
(B) Anterograde transport from ER to Golgi
(C) Transport from the TGN to the vacuole
(D) Endocytosis at the plasma membrane

2010

**208.** The tonoplast membrane is highly specialized to withstand and maintain which of the following physical conditions?
(A) Proton gradients and high internal turgor
(B) Complete osmotic equilibrium with the apoplast
(C) A strictly neutral pH
(D) A uniform lipid composition identical to the plasma membrane

2022

**209.** The C2 photorespiratory cycle involves the transamination of glyoxylate to glycine. This specific enzymatic step occurs within the:
(A) Peroxisome
(B) Chloroplast stroma
(C) Mitochondrial matrix
(D) Cytosol

2009

**210.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Asymmetric cell division is an absolute requirement for the initiation of stomatal lineage in the plant epidermis.
Reason (R): Asymmetric division partitions fate-determining transcription factors unequally, yielding a meristemoid and a larger pavement cell.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**211.** Which primary cytoskeletal network is heavily utilized by myosin motors to drive cytoplasmic streaming?
(A) F-actin microfilaments
(B) Cortical microtubules
(C) Spindle microtubules
(D) Nuclear lamins

2012

**212.** If a cell fails to properly replicate its DNA during S phase, the DNA damage checkpoints will prevent entry into mitosis by inhibiting the activation of:
(A) Mitotic CDKs like CDK1/Cdc2 equivalents
(B) G1 cyclins
(C) CDK4/6 homologs
(D) DNA polymerase

2017

**213.** The irreversible nature of cell cycle phase transitions is guaranteed biochemically by the:
(A) Targeted ubiquitin-mediated cyclin proteolysis

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(B) Continuous synthesis of new DNA
(C) Reversible phosphorylation of structural proteins
(D) Slow diffusion of transcription factors

2008

**214.** During prometaphase, the sudden breakdown of the nuclear envelope in open mitosis allows:
(A) Spindle microtubules to capture kinetochores
(B) Ribosomes to enter the nucleus
(C) Chromosomes to rapidly decondense
(D) Homologous recombination to begin

2016

**215.** Vesicle fusion at the cell plate is precisely mediated by specific fusion machinery, particularly involving complexes of:
(A) SNARE proteins
(B) Porins
(C) Clathrin triskelions
(D) Importins

2014

**216.** The specific point of genetic crossover visible during late prophase I is the chiasma. Chiasmata are essential not only for genetic variation but also for:
(A) Holding homologous chromosomes until anaphase I
(B) Ensuring sister chromatids separate early
(C) Dissolving the nuclear envelope
(D) Assembling the cell plate

2016

**217.** Meiosis II lacks a preceding S phase. This unique feature directly results in:
(A) Ensuring the reduction of DNA content to reach the final haploid state of the gametes
(B) The doubling of the DNA content
(C) Extensive homologous recombination
(D) A failure to separate sister chromatids

2021

**218.** The signal hypothesis fundamentally solved the biological problem of how:
(A) Proteins are directed to subcellular compartments
(B) Cells recognize extracellular hormones
(C) DNA replication is initiated
(D) Chloroplasts harvest light energy

2016

**219.** The TOC translocon located on the outer chloroplast envelope specifically recognizes:
(A) N-terminal transit peptides
(B) C-terminal PTS1 signals
(C) Internal NLS sequences
(D) KDEL retention motifs

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2003

**220.** Match the types of plastids in List I with their primary distinguishing functional characteristic in List II.
List I: (Types of Plastids)
P. Etioplast
Q. Amyloplast
R. Chromoplast
S. Elaioplast
List II: (Primary Distinguishing Functional Characteristic)
I. Starch storage for gravitropism
II. Prolamellar body formation in darkness
III. Lipid/oil storage
IV. Non-photosynthetic pigment accumulation
(A) P-II, Q-I, R-IV, S-III
(B) P-I, Q-II, R-III, S-IV
(C) P-II, Q-IV, R-I, S-III
(D) P-III, Q-I, R-IV, S-II

2025

**221.** The transport of an importin-cargo complex into the nucleus terminates when the complex encounters:
(A) Nuclear Ran-GTP binding to release cargo
(B) A high concentration of Ran-GDP
(C) The nucleolus
(D) The inner nuclear membrane lipid bilayer

2010

**222.** The transition of a somatic plant cell to an embryogenic state (totipotency) typically requires a preliminary phase of:
(A) Dedifferentiation marked by chromatin remodeling
(B) Rapid terminal differentiation
(C) Extensive programmed cell death
(D) Total loss of the central vacuole

2022

**223.** The senescence of a leaf is considered a highly ordered developmental process rather than necrosis because:
(A) Active nutrient salvage prior to cell death
(B) It involves uncontrolled plasma membrane rupture
(C) It is strictly induced by acute freezing injury
(D) DNA is replicated extensively before death

2002

**224.** Which structural feature defines the nucleoid of a prokaryotic cell as fundamentally different from a eukaryotic nucleus?

(A) It completely lacks a bounding lipid bilayer membrane
(B) The DNA is tightly wrapped around an octamer of histones
(C) It contains exactly two pairs of linear chromosomes
(D) It serves as the primary site of ribosome storage

2019

**225.** Secondary cell walls are significantly more rigid and impermeable than primary walls predominantly due to the heavy deposition of:
(A) Lignin
(B) Hemicellulose
(C) Pectin
(D) Callose

2004

**226.** Viral movement proteins facilitate the spread of systemic viral infections primarily by:
(A) Enlarging the size exclusion limit of plasmodesmata
(B) Destroying the middle lamella
(C) Indulating immediate cellular necrosis
(D) Blocking all symplastic transport to trap healthy cytoplasm

2002

**227.** The ratio of saturated to unsaturated fatty acids in plasma membrane phospholipids directly influences:
(A) Phase transition temperature and membrane fluidity
(B) The specific activity of DNA polymerase
(C) The thickness of the primary cell wall
(D) The size of the nuclear pore complex

2023

**228.** Aquaporins enable the rapid transport of water, but rigorously exclude protons ($H^+$) to maintain the electrochemical gradient. This exclusion is achieved through:
(A) Specific pore structure with conserved NPA motifs
(B) An active ATP-hydrolysis mechanism
(C) Continuous flipping of the aquaporin protein
(D) Binding protons permanently to the channel opening

2005

**229.** Arrange the following endomembrane system components in the correct sequence for the secretion of a cell wall protein:
I. Trans-Golgi Network
II. Plasma Membrane
III. Rough Endoplasmic Reticulum
IV. Cis-Golgi Network
Which of the following is the correct sequence?
(A) III, IV, I, II
(B) IV, III, I, II
(C) III, I, IV, II
(D) IV, I, III, II

2006

**230.** Order the steps of nuclear import mediated by the Ran-GTPase cycle:
I. Importin binds to the NLS of the cargo protein in the cytosol.
II. Importin-cargo complex translocates through the NPC.
III. Ran-GTP binds to importin, causing cargo release in the nucleus.
IV. Importin-Ran-GTP complex is exported back to the cytosol.
V. Ran-GAP stimulates GTP hydrolysis, releasing importin.
Which of the following is the correct sequence?
(A) I, II, III, IV, V
(B) I, III, II, IV, V
(C) II, I, III, V, IV
(D) I, II, IV, III, V

2002

**231.** The primary structural components of the thylakoid membrane that directly participate in energy transduction are:
(A) Pigment-protein complexes and ATP synthase
(B) Cellulose synthases
(C) Importins and Exportins
(D) Ribosomes and nucleoids

2023

**232.** The process of protein folding in the ER lumen is highly dependent on an oxidizing environment, which facilitates the formation of:
(A) Disulfide bonds
(B) Peptide bonds
(C) Glycosidic bonds
(D) Phosphodiester bonds

2008

**233.** The targeting of hydrolytic enzymes to the plant vacuole generally involves specific sorting receptors located in the:
(A) Trans-Golgi network
(B) Rough ER
(C) Plasma membrane
(D) Outer mitochondrial membrane

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2009

**234.** Turgor pressure within a plant cell is physically exerted by the vacuole against the plasma membrane, which is ultimately constrained by the:
(A) Cell wall
(B) Tonoplast
(C) Cytoskeleton
(D) Endoplasmic reticulum

2006

**235.** The coordination between peroxisomes, chloroplasts, and mitochondria during photorespiration emphasizes the importance of:
(A) Close apposition and metabolite exchange between organelles
(B) Cellular totipotency
(C) Symplastic isolation
(D) Completely independent organellar function

2003

**236.** The glyoxylate cycle effectively bypasses the two decarboxylation steps of the TCA cycle, allowing a net synthesis of:
(A) Malate from two molecules of acetyl-CoA
(B) $CO_2$
(C) Six-carbon sugars directly within the glyoxysome
(D) ATP

2016

**237.** In highly vacuolated plant cells, which structure forms prior to the preprophase band to suspend the nucleus in the center of the cell?
(A) The phragmosome
(B) The cell plate
(C) The tonoplast
(D) The apoplast

2002

**238.** A critical function of the G1 phase of the cell cycle is the:
(A) Cellular component synthesis and growth assessment
(B) Rapid separation of sister chromatids
(C) Duplication of the chromosomal DNA
(D) Assembly of the phragmoplast

2019

**239.** The activity of the APC/C during late mitosis directly ensures that the cell:
(A) Irreversibly exits mitosis by degrading M-phase cyclins
(B) Replicates its DNA
(C) Remains perpetually in metaphase
(D) Halts cytokinesis

2016

**240.** The separation of sister chromatids at the onset of anaphase is specifically triggered by the cleavage of cohesin molecules by:
(A) Separase
(B) Securin
(C) Cyclin B
(D) DNA polymerase

2012

**241.** During late plant cytokinesis, the vesicular membrane forming the cell plate ultimately fuses with the:
(A) Parental plasma membrane
(B) Nuclear envelope
(C) Tonoplast
(D) Outer chloroplast membrane

2013

**242.** Consider the following statements about meiotic stages:
Pachytene is defined morphologically by the presence of:
(A) Unpaired homologous chromosomes
(B) Fully synapsed bivalents appearing as thick threads
(C) Disassembling spindle fibers
(D) Re-forming nuclear envelopes

2016

**243.** Meiotic recombination (crossing over) is fundamentally essential for:
(A) Novel allele combinations and proper segregation
(B) Increasing the speed of cell division
(C) Duplicating the overall genome size
(D) Suppressing harmful dominant mutations

2012

**244.** The hydrophobic core of a nascent signal peptide is optimally designed to interact directly with:
(A) The 54 kDa subunit of the signal recognition particle
(B) The aqueous environment of the cytosolic domain
(C) Nuclear lamins within the interphase nucleoplasm
(D) The inner lumen of the cis-Golgi apparatus stack

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2011

**245.** Mitochondrial preproteins generally utilize ATP hydrolysis and the membrane potential primarily at which stage of import?
(A) Inner mitochondrial membrane TIM-mediated translocation

(B) Initial recognition by cytosolic chaperones
(C) Cleavage of the transit peptide
(D) Folding within the matrix

**2002**

**246.** Nuclear localization signals (NLS) are typically characterized by a short stretch of:
(A) Basic amino acids like Lysine/Arginine
(B) Highly hydrophobic amino acids (Leucine/ Isoleucine)
(C) Negatively charged amino acids (Aspartate/ Glutamate)
(D) Aromatic amino acids (Phenylalanine/ Tyrosine)

**2015**

**247.** The ability of protoplasts to regenerate a whole plant strictly relies on their inherent:
(A) Totipotency and cell wall regeneration capacity
(B) Vacuolar collapse
(C) Absence of a nucleus
(D) Suppressed cell cycle machinery

**2008**

**248.** The progressive loss of chlorophyll, degradation of macromolecules, and eventual tonoplast rupture are sequenced events defining:
(A) Developmental leaf senescence (a form of PCD)
(B) Early embryonic development
(C) Apical meristem function
(D) Guard cell turgor regulation

**2014**

**249.** The sedimentation coefficient of eukaryotic cytoplasmic ribosomes is 80S, which is composed of:
(A) 50S and 30S subunits
(B) 60S and 40S subunits
(C) 40S and 20S subunits
(D) 70S and 10S subunits

**2017**

**250.** Which layer of the plant cell wall is considered the most biochemically plastic, allowing for substantial surface area expansion during cell growth?
(A) The primary cell wall
(B) The heavily lignified secondary wall
(C) The middle lamella once fully calcified
(D) The suberized casparian strip

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# ANSWERS

**1.** (A) The sedimentation coefficient and distinct rRNA species

Organellar ribosomes in eukaryotes, specifically those found in mitochondria and chloroplasts, are structurally distinct from their cytoplasmic counterparts. They typically exhibit a sedimentation coefficient of 70S, reflecting their endosymbiotic origins. This characteristic, along with specific rRNA species, allows for fundamental differentiation from the 80S eukaryotic cytoplasmic ribosomes while sharing similarities with bacterial prokaryotic ribosomes.

**2.** (C) Cellulose microfibrils embedded in hemicellulose

The primary plant cell wall is a dynamic structure essential for providing mechanical strength and regulating expansion. Its primary framework consists of cellulose microfibrils that are synthesized at the plasma membrane. These microfibrils are intricately embedded within a matrix of hemicellulose and pectins, creating a robust yet flexible network that allows the cell to grow and develop.

**3.** (A) They actively increase the size exclusion limit to facilitate macromolecular transport

Viral movement proteins are specialized factors produced by plant viruses to facilitate their spread through the symplastic network. They interact directly with the structure of plasmodesmata, actively increasing the size exclusion limit of these channels. This modification allows the passage of large viral genomic complexes or whole particles between adjacent cells, effectively bypassing the normal restrictive barriers.

**4.** (D) Sterols and sphingolipids

According to the fluid mosaic model, plant plasma membranes contain distinct microdomains known as lipid rafts. These domains are characterized by a specific lipid composition, predominantly featuring a high concentration of sterols and sphingolipids with long, saturated acyl chains. These lipids pack tightly together, creating stable, ordered platforms that facilitate the clustering of signaling proteins and receptors.

**5.** (B) The electrochemical gradient established by $H^+$-ATPases

Secondary active transport in plants is driven by the potential energy stored in electrochemical gradients rather than direct ATP hydrolysis. Plasma membrane $H^+$-ATPases actively pump protons into the apoplast, creating a steep $H^+$ gradient. This gradient provides the necessary driving force for the movement of other solutes, such as sugars and ions, across the membrane via symporters and antiporters.

**6.** (C) Localized on the inner mitochondrial membrane

The plant mitochondrion contains a complex inner membrane system characterized by numerous invaginations called cristae. This inner membrane serves as the primary site for oxidative phosphorylation. The ATP synthase complex, which synthesizes ATP from ADP and inorganic phosphate, is localized within this membrane. It utilizes the proton motive force generated by the electron transport chain to power its catalytic activity.

**7.** (D) Etioplasts

In the absence of light, proplastids in developing seedlings differentiate into etioplasts instead of chloroplasts. Etioplasts are characterized by the presence of a unique semicrystalline structure known as the prolamellar body. This structure contains precursors for chlorophyll and thylakoid membranes. Upon exposure to light, etioplasts rapidly transform into functional chloroplasts, facilitating the onset of photosynthesis and normal plant growth.

**8.** (A) The rough endoplasmic reticulum

The unfolded protein response is a critical cellular stress mechanism triggered by the accumulation of misfolded or unfolded proteins. This process primarily occurs within the lumen of the rough endoplasmic reticulum, where protein folding and quality control take place. Activation of the UPR leads to the upregulation of chaperones and signaling pathways that aim to restore proteostasis and cellular health.

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**9.** (B) COP-II
Anterograde transport within the endomembrane system involves the movement of proteins and lipids from the rough endoplasmic reticulum to the cis-Golgi network. This process is specifically mediated by COP-II coated vesicles. These vesicles assemble at specialized exit sites on the ER membrane, packaging cargo for transport. Once formed, they bud off and move along the cytoskeleton to reach the Golgi.

**10.** (A) Both A and R are true and R is the correct explanation of A.
The central vacuole is a major organelle that occupies a significant volume in mature plant cells, directly facilitating cell expansion. Tonoplast transporters actively accumulate inorganic ions and organic solutes within the vacuolar lumen. This accumulation creates a lower osmotic potential, driving water influx and generating high turgor pressure. This pressure pushes against the cell wall, allowing for expansion.

**11.** (A) Catalase
Peroxisomes are specialized metabolic organelles involved in diverse oxidative processes, including photorespiration and fatty acid β-oxidation. A defining biochemical feature of these organelles is the presence of high concentrations of the enzyme catalase. Catalase plays a vital role by rapidly neutralizing hydrogen peroxide, a toxic byproduct of oxidative reactions, into water and molecular oxygen, thereby protecting the cell from damage.

**12.** (C) Isocitrate lyase and malate synthase
During the germination of oilseeds, glyoxysomes facilitate the conversion of stored lipids into carbohydrates through the glyoxylate cycle. This pathway is a modification of the TCA cycle that allows for the net synthesis of glucose. Two enzymes unique to this cycle are isocitrate lyase and malate synthase. These enzymes enable the bypass of decarboxylation steps, facilitating nutrient mobilization.

**13.** (D) Microfilaments and myosins
Cytoplasmic streaming, or cyclosis, is the rapid movement of the cytosol and organelles within plant cells. This process is essential for the efficient distribution of nutrients and metabolites. It is primarily driven by the dynamic interaction between actin microfilaments and myosin motor proteins. Myosins move along the actin tracks, generating the mechanical force necessary to propel the cytoplasmic contents.

**14.** (B) G1/S transition
The G1/S transition represents a critical regulatory point in the eukaryotic cell cycle, often termed the restriction point. At this stage, the cell monitors for genomic integrity and the presence of sufficient nutrients. A robust DNA damage checkpoint prevents the initiation of DNA replication if the genetic material is compromised. This ensures that only healthy, undamaged DNA is copied.

**15.** (B) Ubiquitinating cyclins and securin for degradation
The Anaphase-Promoting Complex is a highly regulated E3 ubiquitin ligase that plays a fundamental role in driving the cell cycle forward. Its primary biochemical activity involves the polyubiquitination of specific target proteins, such as mitotic cyclins and securin. This tagging marks these proteins for rapid degradation by the 26S proteasome, which is essential for initiating sister chromatid separation.

**16.** (D) Any kinetochore remains unattached to spindle microtubules
The spindle assembly checkpoint is a vital surveillance mechanism that ensures accurate chromosome segregation during mitosis. It prevents the transition from metaphase to anaphase until all chromosomes are properly aligned and attached to the spindle microtubules. The SAC remains active as long as even a single kinetochore is unattached. Once bipolar attachment is achieved, the checkpoint is silenced.

**17.** (A) Callose with cellulose
During cytokinesis in plant cells, the formation of the cell plate marks the initial boundary between daughter cells. This transient structure is initially rich in callose, which provides a flexible matrix for vesicle fusion. As the cell plate matures into a functional primary cell wall, callose is extensively replaced by cellulose microfibrils. This transition increases the structural rigidity and permanence.

**18.** (C) Pachytene
Prophase I of meiosis is a complex stage divided into several sub-phases based on chromosomal behavior. During pachytene, the process of

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synapsis is completed, and the synaptonemal complex reaches its full assembly. This protein structure aligns homologous chromosomes continuously along their entire length. It provides the necessary framework for genetic recombination, where physical exchanges of genetic material occur between non-sister chromatids.

**19.** (B) Metaphase I
Independent assortment is a fundamental genetic principle that results in increased variation among gametes. This phenomenon occurs during metaphase I of meiosis, where homologous chromosome pairs, known as bivalents, align randomly at the equatorial plate. The orientation of each bivalent is independent of the others, ensuring that maternal and paternal chromosomes are distributed randomly to the daughter cells.

**20.** (A) P-IV, Q-III, R-II, S-I
Efficient protein trafficking requires specific targeting components and recognition signals. The Signal Recognition Particle directs ribosomes to the endoplasmic reticulum, while Sec61 forms the essential translocon channel for entry. Nuclear import is facilitated by Nuclear Localization Signals, which tag proteins for transport through the nuclear pore. Additionally, Ran-GTPase serves as the primary regulator, driving the nuclear-cytoplasmic transport cycle.

**21.** (B) TOC followed by TIC
The import of nuclear-encoded proteins into the chloroplast is a highly coordinated process involving specialized translocon complexes. Proteins destined for the stroma first pass through the Translocon at the Outer envelope of Chloroplasts (TOC) and then through the Translocon at the Inner envelope of Chloroplasts (TIC). This sequential pathway ensures that preproteins are correctly identified and transported across both membranes.

**22.** (C) Ran-GTP
Directionality in nuclear-cytoplasmic transport is established by a steep gradient of Ran-GTP across the nuclear envelope. In the nucleus, Ran-GTP binds to importins that have carried cargo inside. This binding induces a conformational change that triggers the dissociation of the importin-cargo complex, releasing the protein. Ran-GTP then facilitates the export of the empty importin back to the cytoplasm.

**23.** (D) Regenerate into a complete and fertile adult plant
Cellular totipotency is a remarkable characteristic of plant cells, distinguishing them from most animal tissues. It refers to the inherent ability of a single, differentiated somatic cell to undergo dedifferentiation and subsequent reprogramming. Under appropriate hormonal conditions in tissue culture, such a cell can re-enter the cell cycle and eventually regenerate into an entire, functional, and fertile adult plant.

**24.** (A) Tonoplast rupture and release of hydrolytic enzymes
Programmed cell death in plants can manifest in different forms, with vacuolar-type PCD being a prominent developmental mechanism. This process is distinctively characterized by the programmed rupture of the tonoplast, the membrane surrounding the central vacuole. Upon collapse, sequestered hydrolytic enzymes are released into the cytoplasm. These enzymes rapidly degrade cellular components, leading to the efficient clearing of the cell.

**25.** (B) Eukaryotic DNA is linear and enclosed by a nuclear envelope
Eukaryotic and prokaryotic organisms exhibit fundamental differences in their genomic organization and compartmentalization. Eukaryotic genomes consist of multiple linear DNA molecules that are associated with histone proteins and organized into chromosomes. Crucially, this genetic material is sequestered within a double-membrane nuclear envelope. In contrast, prokaryotes typically possess a single, circular chromosome that is not enclosed by a membrane.

**26.** (C) Middle lamella
During the formation of a new plant cell wall, the middle lamella is the first layer to be synthesized. It originates from the cell plate during cytokinesis and acts as an adhesive layer that binds adjacent cells together. This structure is primarily composed of pectic polysaccharides, which can be cross-linked by calcium ions to provide mechanical stability and regulate adhesion.

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**27.** (C) Primary plasmodesmata formed post-cytokinesis across walls
Plasmodesmata are essential channels that facilitate communication between plant cells. Primary plasmodesmata are unique because they are formed during the process of cytokinesis, specifically as the cell plate is being established. In contrast, secondary plasmodesmata can be created de novo across existing cell walls after division is complete. Other components include the desmotubule, a constricted strand of endoplasmic reticulum.

**28.** (A) Increasing the proportion of cis-unsaturated fatty acids
Maintaining membrane fluidity is essential for plant survival during environmental stress, particularly at low temperatures. In response to cold, plants adjust the lipid composition of their plasma membranes. They typically increase the proportion of cis-unsaturated fatty acids within the phospholipid bilayer. These unsaturated bonds introduce kinks in the fatty acyl chains, preventing tight packing to lower the phase transition temperature.

**29.** (D) Relies on existing electrochemical gradients
Facilitated diffusion and active transport are both mediated by specific integral membrane proteins, yet they differ fundamentally in their energy requirements. Facilitated diffusion is a passive process where molecules move down their concentration or electrochemical gradient through channels or carriers. It does not require energy input. Conversely, active transport moves solutes against their gradient, necessitating the expenditure of energy.

**30.** (B) I and III
The mitochondrial genome of plants is notably complex and exhibits several distinctive features. It is significantly larger and more variable in size compared to its mammalian counterparts, often containing many non-coding sequences. Furthermore, it contains essential genes that encode specific components of the electron transport chain and ribosomal machinery. Unlike mammalian mitochondria, the plant mitochondrial genome frequently exists as subgenomic molecules.

**31.** (A) Storage of synthesized starch
Amyloplasts are specialized, non-pigmented plastids found predominantly in non-photosynthetic plant tissues, such as roots and tubers. Their primary physiological function is the storage of starch granules, which serve as an important energy reserve for the plant. Beyond storage, amyloplasts in specific locations like the root cap also play a critical role in sensing gravity, acting as statoliths.

**32.** (B) Correct folding of newly synthesized glycoproteins
N-linked glycosylation is a vital post-translational modification that begins in the rough endoplasmic reticulum. This process involves the covalent attachment of an oligosaccharide precursor to specific asparagine residues of newly synthesized polypeptides. This modification is essential for ensuring the correct folding and stability of glycoproteins. It also serves as a critical signal for the quality control machinery.

**33.** (A) Receiving COP-II coated vesicles from the ER
The Golgi apparatus is a highly organized organelle composed of distinct functional compartments. The cis-Golgi network serves as the entry face for proteins and lipids arriving from the rough endoplasmic reticulum. Its primary responsibility is the reception of COP-II coated vesicles, which transport newly synthesized materials. Once received, these components are processed and modified as they move forward.

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**34.** (D) Secondary alkaloids and tannins
The central vacuole of a plant cell functions as a versatile storage compartment for various substances. One of its key roles is the sequestration of secondary metabolites, such as alkaloids and tannins. These compounds often possess toxic or deterrent properties that serve to protect the plant from herbivory and pathogen attack. Storing them within the vacuole prevents damage to the cytoplasm.

**35.** (D) Chloroplasts and mitochondria
Photorespiration is a complex metabolic pathway initiated when RuBisCO binds oxygen instead of carbon dioxide. The completion of this cycle requires the highly coordinated interaction of three distinct organelles: chloroplasts, peroxisomes, and mitochondria. During the C2 cycle, metabolites move sequentially between these compartments. For instance, glycolate produced in chloroplasts is transported to

peroxisomes, while glycine to serine conversion occurs in mitochondria.

**36.** (A) Net conversion of fatty acids into carbohydrates
Seed germination, particularly in oil-bearing species, requires the efficient mobilization of stored lipids to support early growth. The glyoxylate cycle is a unique metabolic pathway that facilitates the net conversion of fatty acids into carbohydrates. This process occurs within specialized peroxisomes called glyoxysomes. By bypassing the decarboxylation steps of the TCA cycle, the plant can synthesize glucose.

**37.** (A) Cellulose microfibrils in the cell wall
Cortical microtubules are fundamental components of the plant cytoskeleton that reside just beneath the plasma membrane. They play a critical role in determining cell shape by directing the deposition of cellulose microfibrils in the cell wall. Cellulose synthase complexes move along the plasma membrane, following the tracks established by these microtubules. This orientation ensures controlled and directional expansion.

**38.** (C) Late G1 phase
The restriction point is a major regulatory milestone in the eukaryotic cell cycle, occurring during the late G1 phase. It serves as a decisive phase where the cell evaluates environmental cues, such as nutrient availability and growth factors. Once a cell passes this point, it becomes committed to entering the S phase and completing the round of DNA replication and division.

**39.** (A) They are constitutively active throughout the cell cycle
Cyclin-Dependent Kinases are central regulators of the cell cycle, but their activity is far from constitutive. Instead, they are tightly controlled through several mechanisms. Activation strictly requires the binding of stage-specific cyclins, whose concentrations fluctuate throughout the cycle. Additionally, CDK activity can be further modulated by activating and inhibitory phosphorylations, as well as by the binding of specific CDK inhibitors.

**40.** (A) I and II
Mitosis is a highly orchestrated process designed to ensure accurate chromosome distribution. Sister chromatid cohesion is established during the S phase simultaneously with DNA replication, which is essential for proper alignment later. During mitosis, specialized protein structures called kinetochores assemble on the centromeres, mediating the attachment of chromosomes to the spindle microtubules. Conversely, chromosome condensation peaks during metaphase.

**41.** (D) Golgi apparatus
Cytokinesis in plants occurs through the formation of a cell plate, which eventually develops into a new cell wall. This process is initiated by the targeted transport and fusion of numerous vesicles at the equatorial plane. These vesicles are predominantly derived from the Golgi apparatus and contain essential cell wall precursors, such as pectins and hemicelluloses. These vesicles create the nascent membrane.

**42.** (C) Pachytene
Prophase I of meiosis involves several distinct stages that facilitate genetic recombination between homologous chromosomes. The actual process of crossing over, where non-sister chromatids physically exchange segments of DNA, occurs during the pachytene sub-stage. At this point, the synaptonemal complex is fully formed, holding the homologs in tight alignment. This proximity is essential for enzymatic machinery to catalyze strand exchange.

**43.** (A) Separation of sister chromatids
Meiosis consists of two successive nuclear divisions that reduce the chromosome number by half. Meiosis I is unique because it involves the separation of homologous chromosomes, which reduces the cell from diploid to haploid. In contrast, Meiosis II is mechanically similar to mitosis and is characterized by the separation of sister chromatids. This ensures each gamete contains a single replicated copy.

**44.** (A) Hydrophobic signal peptide on the nascent chain
Co-translational translocation is the primary mechanism by which proteins enter the secretory pathway. This process begins when a ribosome starts translating an mRNA encoding a signal sequence. The signal recognition particle (SRP) binds specifically to this hydrophobic signal peptide as it emerges from the ribosome. This binding temporarily halts translation and targets the ribosome-nascent chain complex to the endoplasmic reticulum.

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**45.** (D) Inner mitochondrial membrane
The import of proteins into mitochondria relies on specialized translocon complexes located in the bounding membranes. The TOM complex facilitates initial passage through the outer membrane. Subsequently, the Translocon at the Inner Mitochondrial membrane (TIM) complex mediates the transport of preproteins across the inner membrane into the matrix. This process is often driven by the electrochemical gradient and requires chaperones.

**46.** (A) Importins and exportins
The Nuclear Pore Complex is the primary gatekeeper for the exchange of materials between the nucleus and the cytoplasm. While small molecules can diffuse freely, the passage of larger macromolecules is a highly regulated process. This transport requires specific receptor proteins known as importins and exportins. These transporters recognize specific targeting signals on the cargo and facilitate movement through the pore.

**47.** (A) Produces daughter cells with different fates
Asymmetric cell division is a fundamental mechanism in plant development that generates cellular diversity. During this process, a mother cell divides to produce two daughter cells that differ significantly in size, cytoplasmic content, or the distribution of regulatory factors. This asymmetry results in the daughter cells adopting distinct developmental fates, which is essential for establishing tissue patterns and cell lineages.

**48.** (A) The central vacuole
During the final stages of leaf development, plants undergo a highly regulated process known as senescence. This phase is characterized by the systematic breakdown of cellular components and the mobilization of nutrients. The central vacuole serves as the primary degradation compartment during this period. It accumulates various hydrolytic enzymes that digest proteins, lipids, and nucleic acids, facilitating the efficient recycling of nitrogen.

**49.** (B) Abundant histone-based nucleosomes
The prokaryotic nucleoid is a specialized region within the cytoplasm where the genetic material is localized. It is characterized by several distinct structural features, including the lack of a bounding nuclear envelope and the presence of a single, covalently closed circular DNA molecule. Unlike eukaryotic chromatin, the DNA in a prokaryotic nucleoid is not organized into nucleosomes using traditional histone proteins.

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**50.** (A) I, II and IV
The plant cell wall is a complex extracellular matrix composed of various polysaccharides and proteins. Lignin is a key component of secondary walls, providing essential mechanical strength and waterproofing. Hemicellulose plays a structural role by cross-linking cellulose microfibrils, creating a robust network. Additionally, the middle lamella is rich in pectic substances that facilitate cell adhesion. Conversely, primary walls are cellulose-rich.

**51.** (A) Created de novo across existing cell walls
Plasmodesmata are intercellular channels that provide symplastic continuity between adjacent plant cells. While primary plasmodesmata are formed during cytokinesis, secondary plasmodesmata are unique because they are created de novo across existing, mature cell walls. This process involves the localized degradation of the wall. These channels allow plants to dynamically adjust their connectivity and communication patterns during development or environmental stress.

**52.** (D) Hydrophobic phospholipid core
The plasma membrane functions as a selectively permeable barrier that regulates the movement of substances into and out of the cell. Its fundamental structure is a phospholipid bilayer, which possesses a central hydrophobic core. This core is composed of fatty acyl chains that are highly resistant to the passage of polar or charged molecules. Consequently, such substances require specialized transport proteins.

**53.** (D) Aquaporins
Although water molecules can slowly diffuse through the lipid bilayer of the plasma membrane, their transport is significantly accelerated by a specialized family of integral membrane proteins called aquaporins. These proteins form highly selective channels that allow water to move rapidly in response to osmotic gradients. Aquaporins play a crucial role in maintaining cellular water balance and regulating turgor pressure.

**54.** (B) Increase surface area for ETC and ATP synthase
The ultra-structure of plant mitochondria is characterized by a double-membrane system. The inner membrane is extensively folded into numerous invaginations known as cristae. These folds serve a critical physiological purpose by dramatically increasing the surface area available for metabolic activities. This expanded area accommodates a high density of electron transport chain complexes and ATP synthase molecules, maximizing oxidative phosphorylation efficiency.

**55.** (B) Chloroplasts
Chloroplasts are the primary sites of photosynthesis in green plants and possess a sophisticated internal membrane system. This system includes the thylakoid membranes, which are organized into flattened sacs called grana. The light-dependent reactions of photosynthesis occur exclusively within these thylakoid membranes, where pigment-protein complexes capture solar energy. While other plastids share a common origin, they lack the fully developed thylakoids.

**56.** (A) Lipid synthesis and calcium sequestration
The smooth endoplasmic reticulum is a specialized region of the endomembrane system that lacks associated ribosomes. Its primary functions in plant cells include the biosynthesis of lipids, such as phospholipids and sterols, which are essential for membrane biogenesis. Additionally, the smooth ER serves as a major intracellular reservoir for calcium ions. By sequestering calcium, it plays a vital signaling role.

**57.** (A) O-linked glycosylation
Glycosylation is a fundamental post-translational modification that alters the structure and function of proteins. While N-linked glycosylation begins in the endoplasmic reticulum, O-linked glycosylation is distinctively finalized within the Golgi apparatus. This process involves the covalent attachment of sugar moieties to the hydroxyl groups of serine or threonine residues. These modifications are essential for protein stability and proper sorting.

**58.** (B) Tonoplast
The central vacuole is a dominant organelle in mature plant cells, performing diverse roles in storage and maintenance of turgor pressure. It is bounded by a specialized single lipid bilayer known as the tonoplast. This membrane contains a high density of transport proteins, including $H^+$-ATPases and ion channels, which regulate the exchange of solutes between the cytosol and lumen.

**59.** (B) The endoplasmic reticulum
The biogenesis of peroxisomes is a dynamic process that involves the contribution of other cellular compartments. According to current models, the initial membrane of a peroxisome is derived from the endoplasmic reticulum through a budding process. Specialized vesicles carry essential membrane proteins to form a pre-peroxisomal compartment. Once established, peroxisomes can also grow and undergo fission within the cell.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Glyoxysomes are essential organelles for the survival of germinating seedlings in oil-bearing plants, as they facilitate the conversion of stored lipids into carbohydrates. This process, known as the glyoxylate cycle, provides a vital source of energy and carbon skeletons before the seedling becomes photosynthetically active. By mobilizing these reserves, glyoxysomes ensure that young plants can support rapid early growth.

**61.** (A) Mechanical strength and nuclear architecture
Intermediate filaments are a diverse class of cytoskeletal elements that provide essential mechanical stability to eukaryotic cells. They are particularly important for maintaining the structural integrity of the nucleus, forming a supportive network called the nuclear lamina. Unlike microtubules and microfilaments, which are involved in transport, intermediate filaments primarily function to resist physical stress and anchor various cellular organelles.

**62.** (B) Spindle assembly and mitotic entry
The G2/M transition is a critical regulatory point that ensures a cell is ready for division. A robust DNA damage checkpoint at this stage monitors for any unrepaired genomic lesions. If the cell fails this checkpoint, the progression into mitosis is immediately halted. This arrest prevents the initiation of spindle assembly and nuclear envelope breakdown, ensuring genomic stability is preserved.

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**63.** (B) Binding complexes to prevent kinase activity
Cyclin-Dependent Kinases are central regulators of the cell cycle, and their activity is precisely modulated by various factors. One key mechanism involves the binding of specialized proteins known as CDK inhibitors. These inhibitors interact directly with the CDK-cyclin complexes, physically blocking their kinase activity. By preventing the phosphorylation of substrates, CKIs act as negative regulators of cell cycle progression.

**64.** (B) Hold sister chromatids together until anaphase
Cohesin is a multi-protein ring complex that plays a fundamental role in maintaining genomic integrity during the cell cycle. Its primary function is to hold sister chromatids together from the time of their synthesis in S phase until their separation during anaphase. This cohesion is essential for ensuring that each pair of chromatids aligns properly on the mitotic spindle.

**65.** (B) Microtubules and actin microfilaments
The phragmoplast is a specialized cytoskeletal structure essential for cytokinesis in land plants. It serves as a scaffold for the assembly of the cell plate at the equatorial plane of the dividing cell. The phragmoplast is predominantly constructed from an organized array of microtubules and actin microfilaments. These elements guide the transport of Golgi-derived vesicles to the cell center.

**66.** (C) Chiasmata
During the diplotene stage of prophase I in meiosis, homologous chromosomes begin to separate but remain connected at specific points. These visible, X-shaped regions are known as chiasmata. They represent the physical manifestations of previous crossing over events, where genetic material was exchanged between non-sister chromatids. Chiasmata play a critical role in ensuring proper chromosome segregation during the first division.

**67.** (A) Random segregation of homologous chromosomes
Independent assortment is a key genetic principle that contributes to the diversity of gametes produced during meiosis. This process is characterized by the random segregation of homologous chromosomes into daughter cells during the first meiotic division. The orientation of each homologous pair at the metaphase plate is independent, meaning that each gamete receives a unique combination of maternal and paternal chromosomes.

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**68.** (B) SRP-nascent peptide binding and ER targeting
The signal hypothesis describes the mechanism by which proteins are targeted to the endoplasmic reticulum during synthesis. This process begins with the emergence of a signal peptide from the ribosome. The signal recognition particle (SRP) binds to this peptide, temporarily pausing translation. The complex is then targeted to an SRP receptor on the ER membrane, where the protein is translocated.

**69.** (A) Stroma
The import of nuclear-encoded proteins into the chloroplast is a highly regulated process involving two major translocon complexes. The TOC complex resides in the outer envelope, while the TIC complex is located in the inner envelope. Together, these complexes facilitate the coordinated passage of preproteins from the cytosol into the chloroplast stroma. Once inside, the transit peptide is typically removed.

**70.** (A) P-II, Q-IV, R-I, S-III
Plant cells utilize diverse structures for communication and transport. Plasmodesmata facilitate symplastic cell-to-cell communication. The Nuclear Pore Complex regulates the exchange of materials between the nucleus and cytosol through importin-mediated transport. Additionally, aquaporins are specialized channels that mediate rapid water movement, while Sec61 is a critical component of the translocon channel involved in the protein translocation into the ER.

**71.** (A) NES (Nuclear Export Signal)
The exchange of macromolecules between the nucleus and the cytoplasm is a selective process governed by specific targeting signals. While nuclear import relies on localization signals, the export of mRNA and ribosomal subunits is directly dependent upon a recognition signal known as the Nuclear Export Signal (NES). This signal is recognized by specialized exportins, which facilitate movement through the nuclear pore.

**72.** (B) Phytohormone-driven reprogramming
The transition from a totipotent state to a specific differentiated cell type is a central process in plant development. This induction typically involves the precise orchestration of complex signaling pathways, which are frequently driven by phytohormones. Changes in the relative levels of auxin and cytokinin can trigger the reprogramming of gene expression patterns. This hormonal control ensures coordinated tissue formation.

**73.** (A) Uncontrolled cell rupture from acute stress
Cell death in plants can be broadly classified into programmed and accidental forms. Necrosis represents an uncontrolled type of cell death that typically results from acute environmental stress or severe injury. Unlike vacuolar-type programmed cell death, which is highly regulated, necrosis is characterized by rapid cell rupture and the leakage of cytoplasmic contents. This sudden collapse leads to inflammatory responses.

**74.** (A) Circular genomes and 70S organellar ribosomes
The endosymbiotic theory provides a widely accepted explanation for the evolutionary origin of mitochondria and chloroplasts. This concept is supported by striking similarities between these organelles and prokaryotes. For instance, both organelles possess their own circular genomes. Furthermore, they contain 70S ribosomes that are structurally more similar to bacterial ribosomes than the 80S cytoplasmic ribosomes found in eukaryotic cells.

**75.** (A) Hydrophobicity and high compressive strength
Lignification is a critical developmental process in the formation of secondary cell walls in vascular plants. The deposition of lignin, a complex phenolic polymer, confers significant physiological advantages. It provides high compressive strength, allowing plants to grow tall. Additionally, lignin's hydrophobic nature renders the wall impermeable to water, which is essential for efficient long-distance transport through the xylem without leakage.

**76.** (B) Cytoplasmic continuum linked by plasmodesmata
The symplast is a fundamental concept in plant physiology, representing a continuous network of living cytoplasm that extends throughout the plant body. This cytoplasmic continuum is established and maintained by plasmodesmata, which are specialized channels that traverse the cell walls of adjacent cells. The symplastic domain allows for the efficient and regulated movement of nutrients and signaling molecules between cells.

**77.** (A) Enrichment in saturated sphingolipids and sterols
Lipid rafts are specialized microdomains within the plant plasma membrane that play a vital role in organizing cellular processes. These domains are hallmarked by a unique lipid composition, specifically being enriched in saturated sphingolipids and sterols. These lipids pack tightly together, creating a more ordered environment compared to the surrounding fluid membrane. This organization facilitates the clustering of signaling proteins.

**78.** (C) ATP-binding and hydrolysis domains
Active transport is essential for maintaining cellular homeostasis by moving solutes against their concentration gradients. Primary active transporters are distinct from passive channels because they directly utilize energy to power transport. To achieve this, these proteins must possess specific structural features, including ATP-binding and hydrolysis domains. The energy released from ATP induces conformational changes allowing solutes to move across membranes.

**79.** (C) Porins (VDACs)
The mitochondria are bounded by a double-membrane system that exhibits different permeability characteristics. The outer mitochondrial membrane is generally much more permeable to small metabolites than the inner membrane. This property is primarily due to the abundant presence of specialized integral membrane proteins known as porins, or VDACs. These proteins form aqueous-filled pores that allow the free diffusion of ions.

**80.** (C) I
Plastids are a diverse family of organelles that can interconvert depending on developmental and environmental signals. Chromoplasts specialize in the synthesis of carotenoid pigments. Leucoplasts are unpigmented plastids involved in various biosynthetic pathways. However, etioplasts do not develop in intense light; instead,

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they are characteristic of seedlings grown in the dark, where they contain unique, semicrystalline prolamellar bodies.

**81.** (A) Asparagine
N-linked glycosylation is a major post-translational modification that occurs within the rough endoplasmic reticulum. This process is essential for the proper folding and stability of many proteins. During this modification, a pre-formed oligosaccharide precursor is covalently attached to a specific amino acid residue on the polypeptide chain. In N-linked glycosylation, this attachment occurs at a specific asparagine residue.

**82.** (C) TGN to the plasma membrane/endosomes
Vesicular trafficking within the endomembrane system is mediated by various protein coats that provide specificity for transport. Clathrin-coated vesicles are predominantly involved in the sorting and transport of proteins between the trans-Golgi network and other destinations, such as the plasma membrane or endosomes. These vesicles play a critical role in both the secretory pathway and regulated endocytosis.

**83.** (D) Regulate pH and maintain cellular turgor
The central vacuole of a plant cell functions as a versatile compartment for the accumulation of various solutes, including organic acids like malate and citrate. This sequestration serves several important physiological purposes. Firstly, it plays a vital role in regulating cytoplasmic pH. Secondly, the high concentration of organic acids contributes to the osmotic potential, helping to maintain cellular turgor pressure.

**84.** (B) Hydrogen peroxide into water and oxygen
Peroxisomes are metabolic organelles that host diverse oxidative reactions, many of which generate hydrogen peroxide as a byproduct. Because hydrogen peroxide is a highly reactive and potentially damaging molecule, its rapid neutralization is critical for health. Catalase is the primary enzyme responsible for this detoxification within the peroxisome. It catalyzes the breakdown of hydrogen peroxide into water and oxygen.

**85.** (A) Succinate and glyoxylate
The glyoxylate cycle is a unique metabolic pathway that allows plants to convert lipids into carbohydrates during seed germination. A key enzyme in this cycle is isocitrate lyase, which is localized within glyoxysomes. This enzyme facilitates a critical step by cleaving isocitrate into two distinct molecules: succinate and glyoxylate. This reaction allows the cycle to bypass the decarboxylation steps.

**86.** (C) Head-to-tail G-actin assembly with ATP
Actin microfilaments are essential components of the eukaryotic cytoskeleton, providing structural support and driving various forms of motility. These filaments are composed of globular actin subunits, known as G-actin. The assembly of these subunits occurs in a specific head-to-tail fashion, which is dependent on the binding of ATP. This directional polymerization imparts a distinct structural polarity to the filament.

**87.** (A) Transcription of S-phase specific genes
The transition from the G1 phase into the S phase is a major commitment point in the eukaryotic cell cycle. This progression is irreversibly triggered by the activity of G1/S-specific cyclin-dependent kinases. These kinases catalyze the hyperphosphorylation of key regulatory proteins. This phosphorylation event releases transcription factors that activate the expression of genes essential for starting DNA replication.

**88.** (A) Targeted ubiquitin-mediated proteolysis
Cyclins are critical regulatory proteins whose concentrations vary significantly throughout different phases of the cell cycle. This oscillation is essential for the timely activation and inactivation of cyclin-dependent kinases. The rapid decline in cyclin levels at specific transition points is achieved through targeted, ubiquitin-mediated proteolysis. This irreversible process ensures that the cell cycle proceeds in a unidirectional and orderly manner.

**89.** (B) Condensin
As a cell prepares for mitosis, its chromatin must undergo extensive condensation to form distinct, manageable chromosomes. This process is essential for the accurate segregation of genetic material. Chromosome condensation is primarily driven by the activity of a specialized multiprotein complex known as condensin. This complex utilizes the energy from ATP hydrolysis to organize and compact the chromosomal DNA.

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**90.** (A) I, II and III
Cytokinesis in plant cells is a highly coordinated process that differs significantly from animal cells. It is directed by the phragmoplast, a specialized cytoskeletal structure that guides vesicles to the division plane. These Golgi-derived vesicles fuse to form the cell plate, which grows centrifugally. During the early stages, callose serves as a key transient component. Cleavage furrows are absent.

**91.** (D) Diakinesis
Prophase I of meiosis is a prolonged and complex stage characterized by chromosomal pairing and recombination. The final sub-stage of this phase is diakinesis. During this period, the homologous chromosomes reach their maximum level of condensation. Crucially, the chiasmata, which were formed during pachytene, begin to resolve. This process involves the progressive movement of the chiasmata towards the ends of chromosomes.

**92.** (C) Meiotic anaphase I
Meiosis is a specialized type of cell division that results in the reduction of chromosome number. A defining feature of the first meiotic division is the segregation of homologous chromosomes, rather than sister chromatids. This unique event occurs during anaphase I, when the spindle fibers pull each member of a homologous pair towards opposite poles. This ensures that resulting cells are haploid.

**93.** (A) N-terminal signal peptide hydrophobicity
The accurate targeting of proteins to the endoplasmic reticulum is essential for the proper functioning of the secretory pathway. This process depends on the recognition of a specific signal sequence located at the N-terminus of the nascent polypeptide chain. The fidelity of this recognition is primarily determined by the hydrophobicity of the signal peptide. The signal recognition particle binds specifically here.

**94.** (D) N-terminal amphipathic α-helix signals
The import of nuclear-encoded proteins into mitochondria is a highly regulated process that begins with recognition at the outer membrane. The TOM complex serves as the primary receptor and translocon for these preproteins. It identifies its targets by interacting with specific structural features, typically an N-terminal transit peptide. These signals often form an amphipathic α-helix characterized by charged residues.

**95.** (C) The Ran-GTPase cycle GTP hydrolysis
The movement of macromolecules between the nucleus and the cytoplasm is an active process that requires energy. This transport is not directly powered by ATP hydrolysis, but rather by the Ran-GTPase cycle. The energy driving the active transport through the Nuclear Pore Complex is ultimately derived from the hydrolysis of GTP by the Ran protein, maintaining cellular spatial organization.

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**96.** (A) Stomatal guard cells
Asymmetric cell division is a fundamental developmental mechanism in plants that generates cellular diversity. This process is most visibly instrumental in the formation of specialized epidermal structures, such as stomatal guard cells. During the development of the stomatal lineage, a series of asymmetric divisions occur, partitioning key transcription factors. These cells eventually differentiate into functional guard cells for gas exchange.

**97.** (A) Vacuolar collapse and cytoplasm clearance
The maturation of tracheary elements in the xylem is a classic example of developmentally programmed cell death in plants. This process is essential for creating water-conducting tubes. The hallmark event defining the execution phase of this PCD is the programmed collapse of the central vacuole. This rupture releases hydrolytic enzymes into the cytosol, which rapidly digest the cytoplasm and internal organelles.

**98.** (A) Internal metabolic compartmentalization
The fundamental distinction between prokaryotic and eukaryotic cellular organization lies in the degree of internal complexity. Eukaryotic cells are characterized by extensive metabolic compartmentalization achieved through membrane-bound organelles, such as the nucleus and mitochondria. These specialized compartments allow for the segregation of diverse biochemical reactions, facilitating increased efficiency and regulatory control, which is a defining hallmark of eukaryotic life.

**99.** (B) CESA complexes along cortical microtubules
The orientation of cellulose microfibril deposition is a critical factor in determining the direction of plant cell expansion. This process

is fundamentally directed by the movement of cellulose synthase (CESA) complexes within the plasma membrane. These complexes are guided along the underlying tracks of cortical microtubules. As they synthesize cellulose chains, their path follows the orientation of the underlying cytoskeleton.

**100.** (A) I, II, III and V
Membrane transport in plants is achieved through various mechanisms that regulate solute movement. Primary active transport directly utilizes ATP, while secondary active transport relies on the energy stored in ion gradients. Passive transport is mediated by channels and carriers, with channels typically allowing faster rates. Facilitated diffusion also requires proteins to assist movement. Aquaporins facilitate passive diffusion, not active transport.

**101.** (A) 1-5 kDa
Plasmodesmata are essential channels that connect the cytoplasm of adjacent plant cells, facilitating the symplastic transport of various molecules. The permeability of these channels is characterized by their size exclusion limit, which defines the maximum molecular mass that can move through freely via simple diffusion. For typical, unmodified primary plasmodesmata, this limit is generally considered to be 1 to 5 kilodaltons.

**102.** (C) Lateral diffusion within the plane of the lipid bilayer
The fluid mosaic model describes the dynamic nature of biological membranes. The concept of membrane fluidity implies that both phospholipids and membrane proteins are not fixed in a rigid structure but are capable of lateral movement within the plane of the lipid bilayer. This lateral diffusion is essential for various cellular functions, including receptor clustering, signaling molecule distribution, and vesicle fusion events.

**103.** (B) Transport is limited by the number of available carrier proteins
Carrier-mediated facilitated diffusion is a selective transport process that exhibits saturation kinetics, similar to enzymatic reactions. This phenomenon indicates that the rate of transport does not increase indefinitely with the concentration of the solute. Instead, the transport rate reaches a maximum when all available carrier proteins in the membrane are occupied. This saturation highlights the role of specialized integral proteins.

**104.** (C) Invaginations known as cristae
The inner mitochondrial membrane is a highly specialized structure essential for energy production. To accommodate the vast assembly of respiratory chain complexes and ATP synthase molecules, this membrane features numerous invaginations known as cristae. These folds significantly increase the total surface area of the inner membrane without increasing volume. This structural adaptation provides a massive platform for efficient oxidative phosphorylation processes.

**105.** (B) The thylakoid membrane system
The development of functional chloroplasts from proplastids is a highly regulated process that is triggered by light perception. This transformation involves massive biochemical and structural changes, most notably the biosynthesis of the complex thylakoid membrane system. During this process, proteins and lipids are synthesized to form the flattened sacs and grana where the light-dependent reactions of photosynthesis eventually occur.

**106.** (A) Upregulating ER-resident chaperones
The unfolded protein response is a vital stress-sensing mechanism that aims to restore homeostasis within the endoplasmic reticulum. When misfolded proteins accumulate, the UPR is activated, leading to a coordinated cellular response. One of the primary ways it restores ER function is by upregulating the expression of ER-resident chaperones. These proteins assist in proper folding or target proteins for degradation.

**107.** (D) Protein sorting into clathrin-coated vesicles
The Golgi apparatus is a polarized organelle where proteins and lipids undergo progressive modification and sorting. While the cis-Golgi network primarily receives materials from the endoplasmic reticulum, the trans-Golgi network (TGN) serves as a major sorting station. A distinct function of the TGN is the packaging of proteins into specialized clathrin-coated vesicles for transport to endosomes or the vacuole.

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**108.** (B) Regulating osmotic balance and rapid water flux across the vacuolar membrane
Tonoplast intrinsic proteins represent a specialized class of aquaporins localized exclusively to the membrane of the central vacuole. These proteins are essential for regulating the rapid flux of water across the tonoplast in response to changes in osmotic pressure. By facilitating efficient water exchange between the cytosol and the lumen, TIPs play a vital role in maintaining cellular turgor pressure.

**109.** (A) Glycolate to glycerate
The C2 photorespiratory cycle is a vital metabolic pathway that recovers carbon lost when RuBisCO oxygenates ribulose-1,5-bisphosphate. This cycle requires the close coordination of chloroplasts, peroxisomes, and mitochondria. The overall process involves the conversion of glycolate, which is produced in the chloroplast, into glycerate through several steps. Glycerate is then returned to the chloroplast to re-enter the Calvin-Benson cycle.

**110.** (A) Both A and R are true and R is the correct explanation of A.
The glyoxylate cycle is a unique metabolic pathway primarily active during the early stages of seedling development, where it facilitates the conversion of stored lipids into carbohydrates. In mature, photosynthetically active leaves, this cycle is typically absent. This is because key enzymes are localized within glyoxysomes, which either degrade or transition into leaf peroxisomes once photosynthesis is successfully established.

**111.** (A) Preprophase band of microtubules
Proper spatial organization is essential for accurate cell division in plants. Prior to mitosis, the cell forms a transient cytoskeletal structure known as the preprophase band. This band consists of a dense ring of microtubules and actin filaments that encircles the cell. The location of the preprophase band accurately predicts the future site of the cell plate and the division plane.

**112.** (C) A complete round of DNA replication
The G1/S transition, often referred to as the restriction point, is a critical regulatory milestone in the eukaryotic cell cycle. At this stage, the cell monitors environmental and internal signals. Bypassing this point represents an irreversible commitment to the cell cycle. Once a cell passes the restriction point, it is destined to undergo a complete round of DNA replication.

**113.** (D) Arrest in metaphase from failed securin loss
The Anaphase-Promoting Complex is a central regulator of the cell cycle, functioning as an E3 ubiquitin ligase. Its activity is essential for the transition from metaphase to anaphase. If a loss-of-function mutation occurs in an APC/C gene, the cell will most likely arrest in metaphase. This occurs because the APC/C cannot ubiquitinate targets like securin, which prevents cohesin cleavage.

**114.** (B) Silences the Spindle Assembly Checkpoint
Accurate chromosome segregation depends on the proper attachment of kinetochores to spindle microtubules. During metaphase, the pulling forces generated by the spindle create tension across the sister chromatids. This tension serves as a vital physical signal that all chromosomes are correctly aligned. Sensing this tension silences the spindle assembly checkpoint, allowing the APC/C to trigger the subsequent onset of anaphase.

**115.** (A) Form a transient matrix before cellulose entry
Cytokinesis in plant cells is characterized by the formation of a cell plate at the division plane. During the early stages of this process, callose synthases are heavily targeted to the developing plate. Callose is a β-1,3-glucan that provides a flexible matrix for the fusion of Golgi-derived vesicles. This transient component provides structural support before being replaced by rigid cellulose.

**116.** (A) Facilitating recombination by aligning homologs
The synaptonemal complex is a specialized proteinaceous structure that forms between homologous chromosomes during prophase I of meiosis. Its primary function is to mediate synapsis, ensuring that homologs are precisely aligned. This tight association provides the necessary framework for genetic recombination, allowing enzymatic machinery to catalyze crossing over. By facilitating this exchange, the complex contributes significantly to genetic diversity and segregation.

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**117.** (A) Homologous chromosomes to separate
Meiosis is a highly regulated process designed to reduce the chromosome number in gametes. Errors in chromosome segregation can lead to aneuploidy, where cells have an abnormal chromosome number. Non-disjunction occurring during meiosis I results from the failure of homologous chromosomes to separate properly during anaphase I. Instead of moving to opposite poles, both members of a pair enter one daughter cell.

**118.** (B) Allow unfolded polypeptide passage through membranes
The Sec61 translocon is a highly conserved protein complex that forms a central pore in the endoplasmic reticulum membrane. This structure is essential for the co-translational translocation of proteins into the ER lumen. The central pore of the Sec61 complex is structurally designed to allow the passage of unfolded polypeptide chains while maintaining the permeability barrier of the lipid membrane.

**119.** (A) Cleaved by processing peptidases upon entry
Chloroplast-targeted proteins are synthesized in the cytosol as preproteins containing an N-terminal transit peptide. This peptide is essential for recognizing the TOC/TIC translocon complexes and facilitating import into the stroma. Upon successful entry, the transit peptide is typically removed. Specialized processing peptidases located within the stroma recognize and cleave the peptide, allowing the protein to fold into its mature conformation.

**120.** (A) P-IV, Q-I, R-II, S-III
Precise regulation of the cell cycle involves various specialized proteins. G1 cyclins activate CDKs to prepare for DNA replication. During mitosis, securin acts as an inhibitor, preventing cohesin cleavage until anaphase. Separase is the enzyme responsible for cleaving cohesin rings. Finally, the APC/C functions as an E3 ubiquitin ligase that targets key regulatory proteins, such as cyclins, for rapid degradation.

**121.** (B) Accommodates fully folded proteins through pores
Eukaryotic cells utilize distinct mechanisms for targeting proteins to different organelles. Translocation into the endoplasmic reticulum typically occurs co-translationally and requires proteins to pass through a channel in an unfolded state. In contrast, nuclear import is fundamentally different because it accommodates fully folded proteins and complexes. This process occurs through the Nuclear Pore Complex and is mediated by specific importin receptors.

**122.** (D) SAG upregulation and chlorophyll loss
Leaf senescence is a highly ordered and genetically programmed process that marks the final stage of leaf development. A molecular hallmark of this phase is the upregulation of senescence-associated genes (SAGs). This process is most visibly characterized by the progressive loss of chlorophyll, as the photosynthetic machinery is dismantled. Salvaged nitrogen and minerals are then transported to other plant parts.

**123.** (A) Ethylene-induced vacuolar rupture
Aerenchyma is a specialized tissue containing large gas-filled spaces that facilitate oxygen transport in plants. The formation of this tissue involves developmental programmed cell death in specific cortical cells. This process is typically triggered by ethylene, which accumulates under low-oxygen conditions. Ethylene signaling leads to the programmed rupture of the central vacuole and the subsequent autolysis of cellular contents, creating air channels.

**124.** (A) The nucleosome
The organization of genetic material differs significantly between prokaryotes and eukaryotes. Eukaryotic DNA is highly compacted and organized into a complex structure known as chromatin. The fundamental unit of this packaging is the nucleosome, which consists of DNA wrapped around histone proteins. In contrast, prokaryotic DNA is typically organized into a simpler nucleoid structure that lacks the regular histone-based nucleosome arrangement.

**125.** (B) Root endodermal cells (Casparian strip)
To regulate the movement of water and minerals into the vascular system, plant roots possess specialized barriers. The Casparian strip is found in the endodermal cell layer. These cells are characterized by the deposition of suberin lamellae within their walls. Suberin is a hydrophobic, waxy polymer that blocks apoplastic transport, forcing solutes to move symplastically across the plasma membrane.

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**126.** (A) Contain a desmotubule derived from the ER during division
Plasmodesmata are essential channels that provide symplastic connectivity between adjacent plant cells. A key distinguishing feature of primary plasmodesmata is that they are formed during the process of cytokinesis. As the cell plate is established, strands of the endoplasmic reticulum are trapped within the wall, forming the desmotubule. In contrast, secondary plasmodesmata are created de novo across existing, mature cell walls.

**127.** (A) Restrict fatty acid movement to prevent excessive fluidity
Maintaining membrane fluidity is essential for cellular function. In plant plasma membranes, sterols play a vital role in buffering changes in fluidity. At high temperatures, sterols act to restrict the movement of fatty acid acyl chains within the phospholipid bilayer. This interaction prevents the membrane from becoming excessively fluid. By stabilizing the liquid-ordered phase, sterols ensure that signaling and transport function efficiently.

**128.** (A) Extrude apoplastic protons to generate gradients
Plasma membrane $H^+$-ATPases are essential primary active transporters in plant cells. They utilize the energy released from ATP hydrolysis to actively extrude protons from the cytosol into the apoplastic space. This process generates a steep electrochemical gradient characterized by an acidic exterior and a negative membrane potential. This gradient provides the necessary driving force for the secondary active transport of numerous solutes.

**129.** (B) Intramolecular recombination yielding subgenomic circles
The mitochondrial genome of plants is notably large and exhibits significant structural complexity. This complexity is primarily driven by a high frequency of intramolecular recombination between repeated sequences within the genome. This process leads to the formation of multiple subgenomic circles. Unlike the relatively stable genomes of mammalian mitochondria, the plant mitochondrial genome exists as a dynamic population of rearranged DNA molecules.

**130.** (A) I and II
Plastids are highly versatile organelles that can undergo various transformations based on developmental signals. Etioplasts rapidly convert to functional chloroplasts upon light perception as the prolamellar body reorganizes. Additionally, as leaves age, chloroplasts can senesce into gerontoplasts, characterized by the breakdown of photosynthetic machinery. Importantly, many plastid transitions are reversible, allowing the organelle to redifferentiate based on the plant's needs.

**131.** (C) Calcium
The endoplasmic reticulum is a versatile organelle that performs diverse functions beyond synthesis. It serves as the primary intracellular reservoir for calcium ions, which are crucial for various signaling pathways. Specialized pumps actively transport calcium from the cytosol into the ER lumen, maintaining high concentrations. In response to specific stimuli, sequestered calcium can be rapidly released into the cytosol, triggering responses.

**132.** (B) Serine and Threonine
Glycosylation is a major post-translational modification that alters the properties of many proteins. While N-linked glycosylation begins in the endoplasmic reticulum, O-linked glycosylation is a distinct process that primarily occurs within the Golgi apparatus. This modification involves the covalent attachment of sugar molecules to the hydroxyl groups of serine or threonine residues. This is essential for protein stability and folding.

**133.** (B) High concentrations of accumulated solutes
The central vacuole is the largest organelle in a mature plant cell, essential for maintaining turgor pressure. This immense size is maintained through the continuous osmotic influx of water. This process is driven by the high concentration of solutes, such as inorganic ions and sugars, that are actively accumulated within the vacuolar lumen. The resulting osmotic gradient draws water inward.

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**134.** (A) Peroxisomes
Fatty acid β-oxidation is a fundamental metabolic process that breaks down lipids to provide energy. In animal cells, this pathway occurs in both mitochondria and peroxisomes. However, in plant cells, the β-oxidation of fatty acids is localized exclusively within specialized peroxisomes, such

as glyoxysomes. This compartmentalization is essential for coordinating lipid mobilization with the glyoxylate cycle in germinating seeds.

**135.** (A) The glyoxysome
During early seedling development, the glyoxysome plays a vital role in mobilizing stored reserves. This specialized peroxisome contains the enzymes for the glyoxylate cycle, which converts fatty acids into carbohydrates. As the seedling grows and develops photosynthetic capacity, its reliance on stored lipids decreases. This transition is marked by a metabolic decline in glyoxysomal activity, as they transition into leaf peroxisomes.

**136.** (A) Cellulose synthase (CESA) complexes
Cortical microtubules are fundamental components of the plant cytoskeleton that act as essential positional cues. They directly guide the movement of cellulose synthase (CESA) complexes within the plasma membrane. As these complexes synthesize cellulose chains, they move along the tracks established by the microtubules. This precise coordination ensures that cellulose microfibrils are deposited in a specific orientation, determining cell expansion.

**137.** (B) S phase
The centrosome, or its functional equivalent in plants, is essential for forming the mitotic spindle. Accurate cell division requires that the microtubule-organizing center is duplicated so each daughter cell receives one. This duplication process occurs during the S phase of the cell cycle, coinciding with DNA replication. This coordinated duplication ensures that the cell can establish a bipolar spindle for segregation.

**138.** (A) Metaphase to Anaphase
The cell cycle is regulated by the periodic synthesis and degradation of cyclins. Cyclin B is a critical component of the M-phase promoting factor. The transition from metaphase to anaphase requires the rapid and targeted destruction of Cyclin B. This process is mediated by the Anaphase-Promoting Complex. The resulting drop in CDK activity is a necessary prerequisite for initiating separation.

**139.** (B) Kinetochores
During mitosis, accurate segregation depends on the physical attachment of chromosomes to spindle microtubules. This attachment occurs at specialized, multiprotein structures known as kinetochores, which assemble on the centromeric region of each sister chromatid. Kinetochores serve as the interface between DNA and the spindle, capturing microtubules and sensing tension. This sensing is essential for silencing the spindle assembly checkpoint.

**140.** (A) I
Cytokinesis in land plants involves the formation of a cell plate rather than a cleavage furrow. This process is directed by the phragmoplast, which expands toward the cell periphery and guides Golgi-derived vesicles to the division plane. Importantly, plant cytokinesis is not initiated by a contractile actin ring, which is the primary mechanism used by animal cells for cytoplasmic division.

**141.** (A) Improper segregation and aneuploidy
Crossing over is a fundamental process during prophase I that results in genetic recombination and provides physical links between homologous chromosomes. These links, visible as chiasmata, are essential for proper alignment at the metaphase plate. If homologous chromosomes fail to undergo crossing over, they cannot be held together effectively as bivalents, often leading to improper segregation and aneuploid gamete formation.

**142.** (B) Mitosis, but occurring in haploid cells
Meiosis consists of two successive rounds of nuclear division. While Meiosis I is unique because it involves the segregation of homologous chromosomes, Meiosis II is mechanically much more similar to mitosis. During the second division, sister chromatids are separated and distributed into daughter cells. However, a key difference is that the cells entering Meiosis II are already haploid, having completed reductive division.

**143.** (A) Signal Recognition Particle (SRP)
The targeting of proteins to the secretory pathway begins when a ribosome starts translating an mRNA encoding a signal sequence. The Signal Recognition Particle (SRP) acts as the primary receptor for the nascent signal peptide. The SRP binds specifically to the hydrophobic core of the signal peptide, facilitating the docking of the ribosome-nascent chain complex to the SRP receptor on the ER.

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**144.** (B) Chloroplast stroma
The import of nuclear-encoded proteins into the chloroplast requires crossing two bounding membranes. This process is facilitated by the TOC and TIC translocon complexes. While the TOC complex handles initial passage through the outer envelope, the TIC (Translocon at the Inner envelope of Chloroplasts) complex is specifically responsible for the subsequent transport of proteins into the stroma for folding and maturation.

**145.** (A) Chromatin-bound Ran-GEF factors
The directionality of nuclear transport is driven by a steep gradient of the Ran protein. A high concentration of Ran-GTP is maintained within the nucleoplasm by the localized activity of regulatory factors. Within the nucleus, Ran-GEF (Guanine Nucleotide Exchange Factor) proteins are associated with chromatin and catalyze the exchange of GDP for GTP on the Ran molecule, ensuring continuous transport.

**146.** (B) Auxin and Cytokinin
Cellular totipotency allows plant cells to regenerate whole organisms. In tissue culture, the induction of this state and subsequent organ formation is highly dependent on the precise manipulation of phytohormone levels. Specifically, the ratio between auxin and cytokinin is a critical determinant. A high auxin ratio typically promotes root formation, whereas a low ratio favors the development of shoots from callus.

**147.** (A) Necrosis and rapid vacuolar rupture
The hypersensitive response is a rapid and localized form of programmed cell death used to defend against pathogens. Upon sensing infection, affected cells undergo dramatic changes to restrict pathogen spread. This process is characterized by a rapid burst of reactive oxygen species and the sudden rupture of the central vacuole. This collapse leads to necrotic-like death, creating a barrier of dead tissue.

**148.** (D) The lack of a nuclear envelope
In prokaryotic organisms, the processes of transcription and translation are not spatially separated. This arrangement allows for coupled gene expression, where ribosomes can begin translating an mRNA molecule while it is still being synthesized by RNA polymerase. This is fundamentally possible because prokaryotes lack a nuclear envelope to sequester genetic material. Both processes reside in the same cytoplasmic compartment for efficient production.

**149.** (A) Tensile strength and growth extensibility
The primary plant cell wall provides structural support while allowing for expansion. This balance is achieved through the interaction of polysaccharides. Cellulose microfibrils provide the basic framework, which is then cross-linked by hemicellulose molecules. This cross-linking directly confers tensile strength to the wall. Furthermore, the nature of these interactions allows for controlled "loosening" during cell growth, providing essential extensibility.

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**150.** (A) I, II, III and V
Plasmodesmata are complex intercellular channels that establish a symplastic continuum. The core structure includes a desmotubule, which is a highly constricted strand of the endoplasmic reticulum. Permeability is dynamically regulated, with callose deposition at the neck region acting as a mechanism to restrict transport. Furthermore, plasmodesmata allow for the movement of essential signaling molecules. Viral movement proteins typically increase the limit.

**151.** (A) Amphipathic lipids driving hydrophobic tails together
The spontaneous formation of a lipid bilayer in an aqueous environment is a fundamental biophysical process driven by the amphipathic nature of phospholipids. These molecules possess a hydrophilic head and two hydrophobic tails. When placed in water, the hydrophobic effect drives the tails to aggregate together, away from water. This arrangement results in a stable bilayer, providing a selectively permeable barrier.

**152.** (B) Facilitated diffusion channels
Aquaporins are a specialized family of integral membrane proteins essential for regulating water movement. Structurally, they form highly selective pores that allow water molecules to pass rapidly across the lipid bilayer. Aquaporins are strictly classified as facilitated diffusion channels because they do not require direct energy input. Instead, they facilitate the movement of water along its concentration or osmotic gradient.

**153.** (A) Select core subunits of oxidase and dehydrogenase
The mitochondrial genome is essential for the organelle's function in energy production. It encodes a specific subset of proteins that are critical for the assembly and operation of the electron transport chain. These typically include core subunits of key complexes, such as cytochrome c oxidase. While most mitochondrial proteins are nuclear-encoded, these organelle-encoded subunits are vital for oxidative phosphorylation.

**154.** (A) Statoliths sedimenting to the lowest point
Amyloplasts are specialized, starch-filled plastids that perform a critical role in the perception of gravity. In the columella cells of the root cap, these organelles function as statoliths. Due to their high density relative to the cytosol, amyloplasts sediment to the lowest point of the cell. This settling triggers a signaling cascade that leads to the asymmetric distribution of auxin.

**155.** (A) Cytosolic 26S proteasome via ERAD pathways
The endoplasmic reticulum possesses a robust quality control system to ensure only correctly folded proteins proceed. Misfolded proteins that cannot be repaired by chaperones are targeted for degradation through ER-associated degradation (ERAD). This pathway involves the translocation of these proteins back into the cytosol. Once in the cytosol, they are polyubiquitinated and subsequently degraded by the 26S proteasome.

**156.** (A) COP-I vesicles
Protein trafficking between the ER and Golgi is a highly regulated and bidirectional process. While anterograde transport moves materials forward, retrograde transport is essential for retrieving resident ER proteins that have escaped. These proteins typically possess a C-terminal retention motif recognized by receptors in the cis-Golgi. They are packaged into COP-I coated vesicles and transported back to the endoplasmic reticulum.

**157.** (A) Proton ($H^+$) gradient
Maintaining low concentrations of toxic ions in the cytosol is essential for plant survival. One key mechanism involves the sequestration of these ions into the central vacuole. Tonoplast antiporters facilitate this process by utilizing the energy stored in the proton gradient across the vacuolar membrane. The antiporter then moves toxic ions into the lumen in exchange for protons moving out.

**158.** (A) Assist in the folding of newly synthesized polypeptides
The Binding Protein (BiP) is a crucial chaperone residing within the lumen of the endoplasmic reticulum. It belongs to the Hsp70 family and plays a fundamental role in protein maturation. BiP's primary function is to assist in the proper folding of newly synthesized polypeptides. By binding to exposed hydrophobic regions, BiP prevents premature aggregation and ensures proteins achieve correct conformations.

**159.** (A) The primary transport sugar for the growing axis
During the germination of oilseeds, the mobilization of stored lipid reserves is essential for supporting seedling growth. This process involves the conversion of fatty acids into sucrose. Sucrose is a critical metabolic product because it serves as the primary transport sugar. It can be easily exported from storage tissues and moved through the phloem to provide energy for the growing axis.

**160.** (C) A is true but R is false.
The orientation of newly synthesized cellulose microfibrils in the plant cell wall generally aligns with the orientation of the underlying cortical microtubules. This alignment is critical for determining the direction of expansion. However, the microtubules do not physically transport the microfibrils across the membrane. Instead, they act as tracks that guide the movement of cellulose synthase complexes within the plasma membrane.

**161.** (A) M-phase CDK complexes
The eukaryotic cell cycle is equipped with several surveillance mechanisms known as checkpoints that ensure genomic integrity. The DNA damage checkpoint in the G2 phase is particularly critical, as it prevents entry into mitosis if genetic material is compromised. A persistent break will trigger a signaling cascade that ultimately inhibits the activation of M-phase CDK complexes, preventing the segregation of damaged chromosomes.

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**162.** (B) The APC/C E3 ubiquitin ligase
The timely progression of the cell cycle depends on the precise and irreversible degradation of key regulatory proteins. Cyclins A and B are critical for driving mitosis, but their levels must fall rapidly for anaphase to occur. This decrease is achieved through targeted proteolysis. The Anaphase-Promoting Complex (APC/C) directly tags these cyclins with polyubiquitin chains, marking them for destruction.

**163.** (A) Unattached kinetochores
The spindle assembly checkpoint (SAC) is a vital mechanism that ensures accurate chromosome segregation by preventing premature entry into anaphase. This checkpoint relies on the generation of an inhibitory signal that originates from unattached kinetochores. As long as any kinetochore lacks proper attachment, it recruits signaling proteins, including Mad2. These proteins inhibit the Anaphase-Promoting Complex, preventing subsequent sister chromatid separation.

**164.** (A) Centrifugal, growing outward from the center of the cell
Cytokinesis in land plants is a specialized process that differs fundamentally from the centripetal cleavage observed in animal cells. Instead of pinching the cell, plants build a new boundary from the inside out. This centrifugal process is initiated at the center by the formation of a cell plate. Guided by the phragmoplast, vesicles fuse and expand the plate toward the periphery.

**165.** (B) Nuclear envelope via telomeres
During the early stages of prophase I, homologous chromosomes must undergo extensive movements within the nucleus to find their partners. These movements are essential for recombination and segregation. Chromosomal movements are facilitated by the attachment of the telomeres to the inner surface of the nuclear envelope. Through interactions with the cytoskeleton, the chromosomes are actively shuffled into organized proximity for synapsis.

**166.** (B) Max bivalent condensation and nucleolus dispersal
Diakinesis is the final sub-stage of prophase I and represents a period of significant structural change. This stage is most clearly defined by the homologous chromosome pairs, or bivalents, reaching their maximum level of condensation. Additionally, diakinesis is characterized by the dispersal of the nucleolus and the beginning of nuclear envelope breakdown. The chiasmata move towards the ends of the chromosomes.

**167.** (A) Callose wall
In the anthers of flowering plants, microsporogenesis involves meiotic divisions that produce haploid microspores. Following the completion of the second meiotic division, the resulting four haploid cells, known as a tetrad, are not immediately independent. Instead, they are generally enclosed within a common, thick wall made of callose. This wall provides structural support until enzymatic degradation eventually releases the microspores.

**168.** (A) Signal peptidase
Proteins destined for the secretory pathway are synthesized with an N-terminal signal peptide that directs them to the endoplasmic reticulum. Once the nascent protein begins to enter the lumen, the signal peptide has fulfilled its targeting function. It is then typically removed to allow correct folding. This cleavage is performed by a specialized enzyme complex known as signal peptidase.

**169.** (A) Amphipathic $\alpha$-helices with positive residues
The import of nuclear-encoded proteins into mitochondria is a highly specific process. Most mitochondrial preproteins possess an N-terminal targeting signal often forming an amphipathic $\alpha$-helix. This helix is characterized by having one face rich in positively charged amino acids and the opposite face composed of hydrophobic residues. This structural motif is essential for recognition by the TOM complex for driving translocation.

**170.** (A) P-I, Q-II, R-IV, S-III
Protein trafficking to organelles is governed by specific targeting signals. Chloroplast-bound proteins possess an N-terminal transit peptide. Nuclear transport relies on Nuclear Localization Signals (NLS), typically rich in basic amino acids. Proteins that remain within the endoplasmic reticulum often possess a C-terminal KDEL retention sequence. Additionally, many mitochondrial proteins are directed by an amphipathic presequence that forms a charged $\alpha$-helix.

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**171.** (A) Nucleoporin FG-repeats (Phe-Gly)
The transport of macromolecules through the Nuclear Pore Complex is a highly regulated process mediated by specialized receptor proteins. Importin-β is a key member responsible for ferrying cargo into the nucleus. To move through the pore, importin-β interact directly with the NPC framework. It does this by binding transiently to specific motifs within the nucleoporin proteins, known as FG-repeats.

**172.** (A) Epigenetic and hormonal-signal reversible
Cellular totipotency is a remarkable property of plant cells, implying that the process of differentiation is not necessarily irreversible. This flexibility is largely due to the fact that differentiation involves epigenetic modifications and changes in gene expression patterns. Under appropriate hormonal signals and environmental cues, these epigenetic marks can be remodeled, allowing a specialized cell to dedifferentiate and regenerate new tissues.

**173.** (A) Tonoplast collapse and hydrolytic release
The formation of functional xylem tracheary elements is a classic example of developmentally programmed cell death. This process is essential for creating hollow tubes for water transport. The final execution phase of this PCD is triggered by the programmed collapse of the tonoplast. This rupture releases sequestered hydrolytic enzymes into the cytosol, which rapidly autolyze the cytoplasm and internal cellular organelles.

**174.** (A) Coat protein-mediated vesicle budding and fusion
The fundamental distinction between prokaryotic and eukaryotic cells lies in internal organization. Eukaryotic cells exhibit high biochemical complexity through membrane-bound organelles. A process that distinctly highlights this complexity is the use of coat protein-mediated vesicle budding and fusion for transport. Unlike prokaryotes, eukaryotes utilize specialized protein complexes, such as clathrin, to sort cargo and transport it between various cellular compartments.

**175.** (A) The distribution of cortical microtubules
During the maturation of xylem vessel elements, the deposition of secondary cell walls occurs in specific patterns. These patterns are not random but are directly templated by the organization of the plant's cytoskeleton. Specifically, the distribution of cortical microtubules just beneath the plasma membrane determines where cellulose synthase complexes are targeted. This precise orientation directs the synthesis of reinforced wall patterns.

**176.** (A) Desmotubule made of tightly appressed ER lipid
Plasmodesmata are complex intercellular channels that provide symplastic continuity between adjacent cells. A key structural feature of these channels is the desmotubule, which traverses the central pore. The desmotubule consists of a highly constricted strand of the endoplasmic reticulum that is trapped within the wall. It connects the ER networks of neighboring cells, allowing for the exchange of various lipids.

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**177.** (A) Flippases and floppases utilizing ATP
Biological membranes are characterized by a distinct asymmetric distribution of lipids between the inner and outer leaflets. This organization is essential for various cellular processes, including signaling. Because the spontaneous movement of lipids between leaflets is extremely slow, this asymmetry must be actively maintained by specialized proteins. Flippases and floppases utilize energy from ATP hydrolysis to selectively move specific lipids across.

**178.** (A) II, I, III, IV
Secondary active transport is a coordinated process that utilizes energy stored in electrochemical gradients to move solutes. The sequence of events begins with a primary transporter, such as $H^+$-ATPase, which establishes an outward proton gradient. Subsequently, extracellular sucrose and protons bind to a specialized symporter protein. This binding induces a conformational change, allowing both substances to cross into the cell's interior.

**179.** (A) III, V, I, II, IV
Prophase I of meiosis is a complex stage divided into five distinct sub-stages based on chromosomal behavior. The correct developmental sequence begins with leptotene, followed by zygotene, where synapsis is initiated. Next is pachytene, the stage where crossing over occurs between homologous chromosomes. Subsequently, diplotene marks the period where homologs begin to separate. Finally, diakinesis involves maximum condensation for metaphase.

**180.** (A) TCA cycle enzymes (except succinate dehydrogenase)
The plant mitochondrion is organized into several distinct compartments, each hosting specific biochemical pathways. The innermost compartment, known as the mitochondrial matrix, is a protein-rich environment where several key metabolic processes occur. Most notably, the matrix contains the majority of the enzymes required for the TCA cycle. This localization ensures that the products of the cycle are generated near respiratory chain complexes.

**181.** (B) Gravitropism
Amyloplasts are non-pigmented plastids specialized for the synthesis and storage of starch. While they are found throughout the plant, they perform a unique role within the columella cells of the root cap. In this specific tissue, amyloplasts function as statoliths that sense the direction of gravity. Their sedimenting movement is translated into a biochemical signal that directs downward root growth.

**182.** (A) Secretory protein translation in the cytosol instead of ER
The Signal Recognition Particle is a vital component of the cellular machinery responsible for targeting proteins to the secretory pathway. It identifies the signal sequence of nascent polypeptides and directs the ribosome to the ER. If the SRP fails to function, ribosomes synthesizing proteins would remain in the cytosol. Translation would complete in the cytoplasm rather than being co-translational across the membrane.

**183.** (A) The inner mitochondrial membrane
The trans-Golgi network is a major sorting station in the endomembrane system. It is responsible for packaging proteins and lipids into specialized vesicles for transport to various destinations, including the vacuole and the plasma membrane. However, not all organelles receive materials through the TGN. Most proteins destined for the inner mitochondrial membrane are synthesized in the cytosol and imported directly.

**184.** (D) Glutathione or phytochelatins
The central vacuole of a plant cell serves as a vital compartment for the sequestration of various harmful substances. To prevent these toxic compounds from damaging the cytoplasm, they are often chemically modified before transport. A common mechanism involves tagging the toxins with specialized molecules like glutathione. These tags are recognized by specific transporters on the tonoplast, which then pump them in.

**185.** (A) Catalase-mediated peroxide to water/oxygen conversion
Peroxisomes are metabolic organelles that play a central role in oxidative pathways, including the C2 photorespiratory cycle. Many of these reactions involve the direct use of oxygen and generate hydrogen peroxide as a byproduct. To manage these reactive species, peroxisomes contain high concentrations of the enzyme catalase. Catalase effectively neutralizes hydrogen peroxide by catalyzing its rapid breakdown into water and molecular oxygen.

**186.** (B) Converting lipid-derived acetyl-CoA to 4-carbon acids
During the early growth of oilseed seedlings, the glyoxysome facilitates a unique and essential metabolic transformation. These specialized peroxisomes house the enzymes for the glyoxylate cycle, which allows the plant to convert stored lipids into carbohydrates. Specifically, the cycle takes acetyl-CoA and uses it to synthesize four-carbon dicarboxylic acids. This process effectively bypasses the de-carboxylation steps of the TCA cycle.

**187.** (A) Distinct from animal keratin and less prominent
Intermediate filaments are a major component of the eukaryotic cytoskeleton. While they are well-characterized and highly abundant in animal cells, they are generally considered to be less prominent and more diverse in plants. Plant intermediate filaments are structurally distinct from animal counterparts but still play important roles in maintaining nuclear architecture. They function primarily as structural elements that help resist physical stress.

**188.** (B) G0 phase (quiescence)
The cell cycle is highly regulated, but not all cells are actively dividing. In response to environmental signals or developmental cues, a cell may permanently arrest at the G1 restriction point rather than proceeding into the S phase. This quiescent state is classified as the G0 phase. While in G0, the cell remains metabolically

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active and performs specialized functions but does not divide.

**189.** (A) Serine and Threonine
Cyclin-Dependent Kinases are central regulators of the eukaryotic cell cycle, triggering transitions between phases. Their primary biochemical activity is to transfer a phosphate group from ATP to specific target proteins. CDKs belong to the family of serine/threonine kinases, meaning they specifically target the hydroxyl groups of serine or threonine residues. This phosphorylation induces conformational changes that activate or inhibit substrates.

**190.** (A) Depolymerizing microtubules and motor activity
Anaphase is the stage of mitosis characterized by the rapid separation of sister chromatids toward opposite poles. This movement is driven by mechanical forces generated at the kinetochores. These forces are primarily produced by the coordinated activity of motor proteins and the simultaneous depolymerization of the kinetochore microtubules. As the microtubules shorten, they effectively pull the chromatids toward the spindle poles.

**191.** (A) Temporary flexibility and later structural rigidity
During the formation of the plant cell plate, the transition from callose to cellulose is functionally significant. In early cytokinesis, callose is synthesized rapidly to provide a flexible matrix that facilitates vesicle fusion. As the process nears completion, callose is extensively replaced by cellulose microfibrils. Cellulose provides the necessary structural rigidity and mechanical strength to form a permanent and functional wall.

**192.** (A) Homologous chromosomes align precisely along their entire lengths
Zygotene is the second sub-stage of prophase I and is characterized by the initiation of synapsis between homologous chromosomes. During this period, the synaptonemal complex begins to assemble, acting like a molecular zipper. The initiation of this structure ensures that homologous chromosomes align precisely along their entire lengths. This close and accurate association is a prerequisite for subsequent genetic recombination events.

**193.** (D) Number of homologous chromosome pairs (bivalents)
Independent assortment is a fundamental genetic principle that contributes to the variation observed in sexually reproducing organisms. This process occurs during the first meiotic division, where homologous chromosome pairs align randomly at the metaphase plate. The amount of genetic diversity generated is mathematically dependent on the number of homologous chromosome pairs present. This randomness ensures that each offspring inherits unique mixtures.

**194.** (A) Passing polypeptide chains across a lipid bilayer
The Sec61 translocon complex is an evolutionarily highly conserved structure. This conservation reflects its fundamental and indispensable role in cellular life: the translocation of polypeptide chains across or into a lipid bilayer. The core of the complex forms a central pore through which nascent proteins synthesized by the ribosome pass into the ER. This regulated and selectively permeable channel ensures proper processing.

**195.** (A) Cytosolic chaperones such as Hsp70
Most mitochondrial proteins are encoded in the nucleus and synthesized on free ribosomes in the cytosol. For these preproteins to be successfully imported, they must remain in an unfolded state. To prevent premature folding or aggregation, these polypeptides are bound by specialized cytosolic chaperones, such as the Hsp70 family. These chaperones stabilize the nascent chains until they are recognized for import.

**196.** (A) Within the nucleoplasm
The directionality of nuclear-cytoplasmic transport is fundamentally driven by a steep gradient of the Ran protein. This gradient is established by the localized activity of regulatory proteins. Specifically, Ran-GEF is associated with chromatin inside the nucleus, where it catalyzes the regeneration of Ran-GTP from Ran-GDP. Consequently, the concentration of Ran-GTP is highest within the nucleoplasm, which is essential for transport.

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**197.** (A) A small apical cell and a larger basal cell
Asymmetric cell division is a critical process in plant development that establishes the initial polarity of the embryo. This is most visibly demonstrated during the first division of the

zygote. In many species, this division is unequal, resulting in a small apical cell and a larger basal cell. The apical cell goes on to form the embryo, while the basal cell.

**198.** (C) Uncontrolled bursting from acute freezing damage
Programmed cell death is a highly regulated process that plays essential roles in plant development and defense. It is characterized by an organized breakdown of cellular components. In contrast, cell death resulting from acute environmental stress, such as uncontrolled bursting due to freezing, is classified as necrosis. Necrosis is an accidental and unorganized event where cells lose structural integrity and leak.

**199.** (A) Directly across their plasma membrane
Eukaryotic cells utilize specialized organelles called mitochondria to perform oxidative phosphorylation. In contrast, prokaryotic organisms lack these internal compartments but still require a way to produce energy efficiently. Prokaryotes generally perform oxidative phosphorylation directly across their plasma membrane. The electron transport chain complexes are embedded within this membrane, where they establish a proton gradient between the cytoplasm and the periplasmic space.

**200.** (A) Calcium ($Ca^{2+}$)
Pectins are a major class of polysaccharides found in the cell wall and are particularly abundant in the middle lamella. They play a critical role in regulating cell adhesion. This regulation is largely achieved through the ability of pectic chains to form cross-links. Specifically, negatively charged regions of certain pectins can bind to divalent calcium ions, creating a stable gel-like matrix.

**201.** (B) Reversible callose deposition at the neck
Plasmodesmata are dynamic intercellular channels whose permeability is not fixed but can be rapidly adjusted in response to signals. A key structural mechanism for this regulation involves the reversible deposition of callose at the neck region. When a plant experiences stress, callose synthases are activated to produce a plug. Conversely, callose is degraded by specialized enzymes to restore the channel's normal symplastic permeability.

**202.** (A) Rigid lipid raft formation and signaling
Sphingolipids are a diverse and essential class of lipids found in plant plasma membranes. One of their most prominent roles is in the formation and organization of membrane microdomains known as lipid rafts. Due to their saturated fatty acid chains, sphingolipids pack tightly with sterols to create rigid regions. These lipid rafts serve as stable platforms that facilitate signaling protein clustering.

**203.** (C) $H^+$
The plasma membrane of a plant cell acts as an active barrier that maintains specific ionic concentrations. A central player in this process is the $H^+$-ATPase, a primary active transporter. It utilizes the energy from ATP hydrolysis to pump protons out of the cytosol. This continuous extrusion of $H^+$ ions creates a steep concentration gradient and an electrical potential across the membrane.

**204.** (A) Presence in multiple circular and subgenomic copies
The organization of the plant mitochondrial genome is remarkably complex and differs fundamentally from the nuclear genome. While the nuclear genome is organized into linear chromosomes, the mitochondrial DNA typically exists as a dynamic collection of molecules. It is frequently found in multiple copies and can occur as circular structures or smaller subgenomic fragments. This complexity is increased by recombination between repeats.

**205.** (A) Carotenoids and specialized plastoglobuli
The transition of a chloroplast into a chromoplast is a dramatic developmental process observed during fruit ripening. This transformation involves dismantling the photosynthetic machinery and the loss of chlorophyll. Simultaneously, the organelle synthesizes and accumulates high concentrations of non-photosynthetic pigments, primarily carotenoids. These pigments are often stored within specialized lipid droplets known as plastoglobuli, changing the organelle's color to vibrant shades.

**206.** (A) Opening of ligand-gated IP3 receptor channels
The endoplasmic reticulum serves as a major intracellular reservoir for calcium ions. Maintaining a high concentration of calcium within the ER lumen is achieved by active

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pumping. In response to specific extracellular signals, sequestered calcium is rapidly released into the cytosol. This release is primarily triggered by the opening of specialized ligand-gated channels on the ER membrane, such as those activated by IP3.

**207.** (A) Retrograde transport from Golgi back to ER
Vesicular trafficking within the endomembrane system is a highly organized and bidirectional process. While COP-II vesicles facilitate movement from the ER to the Golgi, COP-I coated vesicles are primarily responsible for retrograde transport. This process moves proteins from the Golgi back to the ER. Retrograde transport is essential for recycling membrane components and retrieving resident ER proteins that have escaped forward.

**208.** (A) Proton ($H^+$) gradients and high internal turgor
The tonoplast is the specialized membrane that bounds the central vacuole, playing a vital role in cellular homeostasis. This membrane must be capable of withstanding and maintaining significant physical and chemical gradients. It houses a high density of transporters that actively pump protons into the lumen. Additionally, the tonoplast must resist high internal turgor pressure generated by the osmotic accumulation of solutes.

**209.** (A) Peroxisome
Photorespiration is a complex metabolic pathway that spans multiple organelles. During the C2 cycle, glycolate produced in the chloroplast is transported to the peroxisome. Within this organelle, glycolate is oxidized to glyoxylate. A critical next step is the transamination of glyoxylate to form the amino acid glycine. This specific enzymatic reaction occurs within the peroxisomal matrix, after which glycine moves to mitochondria.

**210.** (A) Both A and R are true and R is the correct explanation of A.
Asymmetric cell division is an absolute requirement for initiating the stomatal lineage in the plant epidermis. During this process, a mother cell divides unequally to produce daughter cells with distinct fates. This asymmetry is driven by the unequal partitioning of fate-determining transcription factors. This division yields a smaller meristemoid and a larger pavement cell, ensuring organized spacing and distribution of stomata.

**211.** (A) F-actin microfilaments
Cytoplasmic streaming is the rapid movement of the cytosol within plant cells, essential for the efficient distribution of nutrients. This process is driven by the dynamic interaction between the cytoskeleton and motor proteins. In plants, the primary cytoskeletal network utilized for this movement is composed of F-actin microfilaments. Specialized myosin motors bind to these actin tracks and propel the cytoplasmic content.

**212.** (A) Mitotic CDKs like CDK1/Cdc2 equivalents
Genomic integrity is essential, and eukaryotic cells have evolved several checkpoints to monitor DNA replication. If a cell fails to properly duplicate its genome, a signaling cascade is activated. This DNA damage checkpoint acts to prevent mitosis entry by inhibiting the activation of mitotic cyclin-dependent kinases. By blocking these master regulators, the cell remains arrested, providing time for DNA repair processes.

**213.** (A) Targeted ubiquitin-mediated cyclin proteolysis
The eukaryotic cell cycle must proceed in a unidirectional and orderly manner. The biochemical guarantee for the irreversible nature of phase transitions is the targeted, ubiquitin-mediated proteolysis of key regulatory proteins. At critical points, specialized E3 ubiquitin ligases tag stage-specific cyclins with polyubiquitin chains. These tagged proteins are then rapidly and permanently degraded by the 26S proteasome, shutting down specific CDK activity.

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**214.** (A) Spindle microtubules to capture kinetochores
In most eukaryotic cells, mitosis is "open," meaning that the nuclear envelope must be dismantled for the spindle to access the chromosomes. This dramatic structural change occurs during prometaphase. The sudden breakdown of the nuclear envelope allows the spindle microtubules to enter the nuclear region. Once inside, they can interact with and capture the specialized protein structures called kinetochores on chromosomes.

**215.** (A) SNARE proteins
Cytokinesis in plant cells is characterized by the formation of a cell plate, requiring the targeted

transport and fusion of vesicles at the division plane. The precise docking and fusion of these vesicles are mediated by a specialized set of proteins known as SNAREs. These proteins form a stable complex that pulls the two lipid bilayers into close proximity, facilitating fusion events.

**216.** (A) Holding homologous chromosomes until anaphase I
During prophase I of meiosis, genetic recombination between homologous chromosomes results in the formation of chiasmata. These visible, X-shaped structures are essential for generating genetic diversity and for the mechanical success of the meiotic process. Chiasmata act as physical links that hold homologous chromosomes together as bivalents. This connection is vital for ensuring homologs align correctly at the metaphase plate.

**217.** (A) Ensuring the reduction of DNA content to reach the final haploid state of the gametes
Meiosis consists of two successive rounds of division, but only one round of DNA replication. Crucially, there is no S phase preceding the second meiotic division. This unique feature is essential for the overall goal of meiosis. By skipping DNA replication between divisions, the cell can reduce its total DNA content by half in the final division, ensuring haploid gamete formation.

**218.** (A) Proteins are directed to subcellular compartments
The signal hypothesis provided a fundamental solution to a major biological question: how are proteins accurately directed to their appropriate subcellular destinations? This theory proposed that proteins destined for the secretory pathway possess a specific amino acid sequence at their N-terminus. This peptide is recognized by specialized cellular machinery which targets the ribosome and nascent protein to the endoplasmic reticulum membrane.

**219.** (A) N-terminal transit peptides
The import of nuclear-encoded proteins into the chloroplast is initiated at the outer membrane by the TOC complex. The TOC complex functions as a gatekeeper, specifically recognizing and binding to the N-terminal transit peptides present on chloroplast-bound preproteins. These transit peptides serve as address labels that ensure only appropriate proteins are imported into the organelle through the double-membrane envelope system.

**220.** (A) P-II, Q-I, R-IV, S-III
Plastids are a versatile family of organelles that differentiate into various forms based on their physiological role. Etioplasts develop in the dark and possess a unique prolamellar body. Amyloplasts are specialized for starch storage and are essential for sensing gravity. Chromoplasts function in non-photosynthetic pigment accumulation, while elaioplasts are specialized for oil storage. These diverse forms allow plastids to perform metabolic functions.

**221.** (A) Nuclear Ran-GTP binding to release cargo
The transport of proteins from the cytoplasm into the nucleus is a highly regulated process. It begins when an importin receptor binds to the Nuclear Localization Signal of a cargo protein. The import process is terminated when the complex reaches the nucleoplasm and encounters high concentrations of Ran-GTP. The binding of Ran-GTP to importin induces a conformational change that releases the cargo.

**222.** (A) Dedifferentiation marked by chromatin remodeling
The transition of a specialized somatic plant cell to an embryogenic or totipotent state is a remarkable process. This transformation typically begins with a preliminary phase of dedifferentiation. During this period, the cell loses its specialized features and re-enters the cell cycle. This process is marked by significant biochemical changes, including extensive chromatin remodeling and shifts in gene expression patterns.

**223.** (A) Active nutrient salvage prior to cell death
Leaf senescence is a highly ordered and genetically programmed developmental process. A key characteristic that distinguishes senescence from necrosis is the active and systematic salvage of nutrients from the aging tissue. Before the cell finally dies, essential elements like nitrogen are retrieved from dismantled proteins and lipids. These salvaged nutrients are then mobilized and transported to other parts of the plant.

**224.** (A) It completely lacks a bounding lipid bilayer membrane
The primary distinction between the prokaryotic nucleoid and the eukaryotic nucleus lies in the degree of structural compartmentalization. The

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prokaryotic nucleoid is a specialized region within the cytoplasm where genetic material is localized. Its most defining feature is that it completely lacks a bounding lipid bilayer membrane. Consequently, the DNA is in direct contact with the cytosol for coupled gene expression.

**225.** (A) Lignin
Secondary cell walls are a critical developmental adaptation providing essential structural support. These walls are significantly more rigid and impermeable than primary walls, a characteristic predominantly due to the heavy deposition of lignin. Lignin is a complex, branched phenolic polymer infused into the wall matrix. This process provides high compressive strength and hydrophobic properties, which are essential for long-distance water transport.

**226.** (A) Enlarging the size exclusion limit of plasmodesmata
To overcome the restrictions of the cell wall and the size exclusion limit of plasmodesmata, many viruses produce specialized movement proteins. These proteins interact directly with the structure of the plasmodesmatal channels, facilitating the passage of viral genomic complexes. Movement proteins achieve this primarily by enlarging the size exclusion limit of the channels, creating a pathway for rapid symplastic spread.

**227.** (A) Phase transition temperature and membrane fluidity
The physical properties of biological membranes are critically dependent on the lipid composition. In particular, the ratio of saturated to unsaturated fatty acids in phospholipid tails directly influences membrane fluidity and phase transition temperature. Saturated fatty acids pack tightly, making membranes rigid, while unsaturated fatty acids increase fluidity. By adjusting this ratio, plants maintain functional membrane integrity across varying environmental temperatures.

**228.** (A) Specific pore structure with conserved NPA motifs
Aquaporins facilitate rapid water transport while rigorously excluding other solutes, including protons. Maintaining this selectivity is vital for preserving electrochemical gradients. Protons are excluded through a specialized pore structure that features highly conserved asparagine-proline-alanine (NPA) motifs. These motifs create a localized electrostatic barrier and specific orientation for water molecules, preventing the formation of a continuous proton wire through the channel.

**229.** (A) III, IV, I, II
The secretion of proteins follows a specific path through the endomembrane system. This sequence begins with synthesis and processing in the rough endoplasmic reticulum. Subsequently, the protein is transported to the cis-Golgi network for initial Golgi processing. After moving through the stacks, the protein reaches the trans-Golgi network for sorting. Finally, secretory vesicles transport the protein to the plasma membrane.

**230.** (A) I, II, III, IV, V
Nuclear import is a regulated cycle ensuring proteins reach the nucleus efficiently. First, an importin binds to the cargo in the cytosol. This complex then translocates through the NPC. Upon reaching the nucleus, the binding of Ran-GTP to importin triggers cargo release. The importin-Ran-GTP complex is then exported back to the cytosol, where GTP hydrolysis occurs to release the importin.

**231.** (A) Pigment-protein complexes and ATP synthase
The thylakoid membrane is a specialized internal membrane system within chloroplasts, serving as the site for light-dependent photosynthetic reactions. Its function depends on key components that participate in energy transduction. These include various pigment-protein complexes, such as the photosystems, which initiate electron transport. Additionally, the thylakoid membrane contains the ATP synthase complex, which utilizes the proton gradient to synthesize ATP.

**232.** (A) Disulfide bonds
The lumen of the endoplasmic reticulum provides an oxidizing environment essential for proper protein folding and maturation. This oxidizing state is critical because it facilitates the formation of disulfide bonds between specific cysteine residues of nascent polypeptide chains. These covalent links are essential for stabilizing the three-dimensional structure of proteins and ensuring they achieve their mature, functional conformation before further secretion.

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**233.** (A) Trans-Golgi network
The targeting of hydrolytic enzymes to the plant vacuole is a highly regulated process. These enzymes are first synthesized in the rough ER and then transported to the Golgi apparatus for modification. The final sorting and packaging of these proteins into specialized vesicles occur at the trans-Golgi network. This sorting process generally involves the recognition of specific signals by vacuolar sorting receptors.

**234.** (A) Cell wall
Turgor pressure is a fundamental physical force in plant cells resulting from the osmotic influx of water into the central vacuole. This pressure is exerted by the expanding vacuole against the plasma membrane, which in turn pushes against the surrounding extracellular matrix. Crucially, this internal force is ultimately constrained by the rigid cell wall, which provides mechanical resistance to prevent bursting.

**235.** (A) Close apposition and metabolite exchange between organelles
Photorespiration is a complex metabolic pathway that involves chloroplasts, peroxisomes, and mitochondria. The successful completion of this cycle depends on the highly coordinated movement of metabolites between these compartments. This interaction is facilitated by the close physical apposition of the organelles within the cytoplasm. Specialized transport proteins on the membranes allow for the efficient exchange of intermediates, maximizing metabolic recycling.

**236.** (A) Malate from two molecules of acetyl-CoA
The glyoxylate cycle is a unique metabolic pathway that allows germinating seedlings to convert stored lipids into carbohydrates. By utilizing two specific enzymes, the cycle effectively bypasses the two de-carboxylation steps of the TCA cycle. As a result, the glyoxylate cycle facilitates the net synthesis of malate and other four-carbon acids from two molecules of acetyl-CoA, providing carbon skeletons for gluconeogenesis.

**237.** (A) The phragmosome
In highly vacuolated plant cells, the nucleus is often located at the cell periphery. To ensure mitosis occurs accurately, the cell must reposition the nucleus centrally. Prior to the preprophase band formation, a specialized structural framework called the phragmosome is established. This structure consists of a sheet of cytoplasm and cytoskeleton that bisects the central vacuole, suspending the nucleus at division.

**238.** (A) Cellular component synthesis and growth assessment
The G1 phase is the first stage of the eukaryotic cell cycle and serves as a period of intense growth. Following cytokinesis, the new daughter cell enters G1 to restore its size and replenish its cellular components. This phase is characterized by the synthesis of RNA and proteins. Crucially, the cell also assesses internal and external environments before committing to DNA replication.

**239.** (A) Irreversibly exits mitosis by degrading M-phase cyclins
The Anaphase-Promoting Complex is a master regulator of the cell cycle. Its activity during late mitosis is essential for the transition to anaphase. By tagging M-phase cyclins with polyubiquitin chains, the APC/C ensures their rapid and irreversible destruction by the proteasome. This drop in cyclin levels leads to the inactivation of mitotic CDKs, which is necessary for mitotic exit.

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**240.** (A) Separase
Accurate segregation of sister chromatids during mitosis is a highly regulated process. Throughout the cell cycle, sister chromatids are held together by a ring complex called cohesin. The separation of these chromatids is specifically triggered by the activity of a protease known as separase. Once the spindle assembly checkpoint is satisfied, active separase allows for the rapid cleavage of the cohesin rings.

**241.** (A) Parental plasma membrane
Cytokinesis in land plants occurs through the formation of a cell plate at the division plane. This process is initiated by the fusion of Golgi-derived vesicles. Guided by the phragmoplast, the cell plate grows centrifugally, extending outward from the center. During the late stages, the vesicular membrane that forms the cell plate eventually reaches and fuses with the parental plasma membrane.

**242.** (B) Fully synapsed bivalents appearing as thick threads
Prophase I of meiosis is a complex stage divided into five sub-phases. The third sub-stage is pachytene, which follows the completion of synapsis. This phase is most clearly defined morphologically by the presence of fully synapsed homologous chromosome pairs, known as bivalents. During pachytene, homologous chromosomes are held in tight alignment by the synaptonemal complex, providing the framework for genetic recombination.

**243.** (A) Novel allele combinations and proper segregation
Meiotic recombination is a fundamental process that involves the physical exchange of genetic material between non-sister chromatids. This process is essential for generating novel combinations of maternal and paternal alleles. Furthermore, the chiasmata formed by recombination provide vital physical links that hold homologous chromosomes together until metaphase I, ensuring their accurate segregation and the reduction of chromosome number in gametes.

**244.** (A) The 54 kDa subunit of the signal recognition particle
Targeting proteins to the secretory pathway is initiated when a ribosome translates a signal sequence. This sequence typically features a characteristic hydrophobic core. As this signal peptide emerges, it is recognized and bound by the Signal Recognition Particle (SRP). Specifically, the hydrophobic core interacts directly with a specialized binding pocket located within the 54 kDa subunit of the complex protein complex.

**245.** (A) Inner mitochondrial membrane TIM-mediated translocation
Import of nuclear-encoded proteins into the mitochondrial matrix is an active process requiring energy. Preproteins utilize ATP hydrolysis and membrane potential primarily during translocation across the inner mitochondrial membrane. This step is mediated by the TIM complex. The electrochemical gradient across the inner membrane provides the driving force, while ATP-dependent chaperones within the matrix help to pull the polypeptide through the channel.

**246.** (A) Basic amino acids like Lysine/Arginine
Nuclear transport is a highly selective process. Most nuclear proteins possess a specific targeting signal known as a Nuclear Localization Signal (NLS). These signals are typically characterized by a short stretch of positively charged, basic amino acids, most commonly lysine and arginine. The NLS is recognized by specialized receptor proteins called importins, which facilitate transport through the Nuclear Pore Complex.

**247.** (A) Totipotency and cell wall regeneration capacity
Protoplasts are plant cells from which the cell wall has been removed. Despite the loss of their structural boundary, protoplasts retain a remarkable ability to regenerate whole plants under appropriate culture conditions. This capacity strictly relies on their inherent cellular totipotency and their ability to rapidly synthesize a new cell wall. Once a protoplast regenerates its wall, it can differentiate.

**248.** (A) Developmental leaf senescence (a form of PCD)
Leaf senescence is the final phase of leaf development, characterized by a series of highly ordered and genetically programmed events. This process is designed to maximize nutrient salvage and recycling. The characteristic sequence includes the progressive loss of chlorophyll as the photosynthetic machinery is dismantled, followed by the systematic degradation of macromolecules. The final execution involves programmed rupture of the tonoplast.

**249.** (B) 60S and 40S subunits
Ribosomes are the essential molecular machines responsible for protein synthesis. In eukaryotic organisms, the cytoplasmic ribosomes are characterized by a sedimentation coefficient of 80S. These ribosomes are complex structures composed of two distinct subunits that differ in size. The large subunit is designated as 60S, while the small subunit is 40S. Each subunit is made up of specific rRNA species.

**250.** (A) The primary cell wall
The plant cell wall is a complex matrix providing structural support. Among the different layers, the primary cell wall is considered the most biochemically plastic. It consists of a flexible network of cellulose microfibrils embedded in hemicelluloses and pectins. This unique

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composition allows the primary wall to undergo significant surface area expansion as the cell grows in response to turgor.

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# CHAPTER 02
# Biomolecules and Metabolism

2001

**1.** Which of the following defines mutarotation in monosaccharides?
(A) The interconversion of epimers in basic solutions
(B) The change in specific rotation accompanying the interconversion of α and β anomers
(C) The formation of glycosidic bonds between two monosaccharides
(D) The conversion of an aldose to a ketose sugar

2016

**2.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): D-glucose and D-mannose are epimers.
Reason (R): They differ only in the stereochemistry at the C-2 position.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**3.** Which qualitative test is specifically used to detect the presence of ketoses?
(A) Benedict's test
(B) Barfoed's test
(C) Iodine test
(D) Seliwanoff's test

2009

**4.** Identify the correct statement regarding anomers.
(A) They are stereoisomers that differ only at the hemiacetal or hemiketal carbon.
(B) They are enantiomers of cyclic monosaccharides.
(C) They have completely different chemical formulas.
(D) They cannot undergo mutarotation in aqueous solutions.

2006

**5.** Which of the following is an INCORRECT statement about epimers?
(A) D-galactose and D-glucose are C-4 epimers.
(B) D-fructose and D-glucose are C-3 epimers.
(C) D-glucose and D-mannose are C-2 epimers.
(D) All epimers are diastereomers.

2012

**6.** Which chemical classification accurately describes ribose?
(A) Aldohexose
(B) Aldopentose
(C) Ketopentose
(D) Ketohexose

2017

**7.** Based on stereochemistry, the naturally occurring biologically active form of most monosaccharides is designated as:
(A) D-form
(B) L-form
(C) Meso form
(D) Racemic mixture

2014

**8.** In Benedict's test for reducing sugars, the visual color change is due to the formation of:
(A) Cupric oxide
(B) Copper sulfate
(C) Cuprous oxide
(D) Copper hydroxide

2016

**9.** Which monosaccharide does NOT possess an asymmetric carbon atom?
(A) Glyceraldehyde
(B) Erythrose
(C) Ribulose
(D) Dihydroxyacetone

2002

**10.** Match the following carbohydrates in List I with their properties in List II.
List I: (Carbohydrates)
P. Maltose
Q. Sucrose
R. Trehalose
S. Lactose
List II: (Properties)
1. Non-reducing, α(1→1)α linkage

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2. Reducing, β(1→4) linkage
3. Reducing, α(1→4) linkage
4. Non-reducing, α(1→2)β linkage

(A) P-III, Q-IV, R-I, S-II
(B) P-IV, Q-III, R-II, S-I
(C) P-III, Q-I, R-IV, S-II
(D) P-II, Q-IV, R-I, S-III

2017

**11.** Which process explains why an aqueous solution of α-D-glucopyranose changes its optical rotation over time?
(A) Mutarotation
(B) Enolization
(C) Racemization
(D) Esterification

2023

**12.** Which type of glycosidic linkage is primarily found in the linear structural polysaccharide cellulose?
(A) α(1→4)
(B) β(1→4)
(C) α(1→6)
(D) β(1→6)

2002

**13.** Identify the correct pair representing a storage polysaccharide and its major branching linkage.
(A) Amylose : α(1→6)
(B) Glycogen : α(1→6)
(C) Cellulose : β(1→4)
(D) Pectin : α(1→2)

2007

**14.** Which polysaccharide is characterized by repeating units of D-galacturonic acid joined by α(1→4) linkages?
(A) Pectin
(B) Hemicellulose
(C) Chitin
(D) Inulin

2005

**15.** Identify the INCORRECT pair regarding polysaccharides.
(A) Starch - Storage in plants
(B) Glycogen - Storage in animals
(C) Hemicellulose - Storage in fungi
(D) Cellulose - Structural in plants

2002

**16.** What is the fundamental functional role of hemicellulose in the plant cell wall?
(A) It forms the primary load-bearing microfibrils within the secondary cell wall.
(B) It acts as a primary energy reserve utilized during the germination of seeds.
(C) It provides localized enzymatic activity for the degradation of the cell wall.
(D) It cross-links cellulose microfibrils to strengthen the cell wall.

2006

**17.** The most appropriate reason for the insolubility of cellulose in water is:
(A) Extensive intra- and inter-molecular hydrogen bonding
(B) Presence of various non-polar hydrophobic side chains
(C) Highly branched and amorphous nature of its overall molecular structure
(D) Lack of available free hydroxyl groups on the constituent glucose monomers

2018

**18.** Which statement about starch is most accurate?
(A) It consists solely of amylopectin molecules regardless of the plant species.
(B) It forms a helical structure that produces a blue-black color with iodine.
(C) It possesses a branched amylose structure while amylopectin remains unbranched.
(D) It is localized primarily within the secondary cell wall of mature plant cells.

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2015

**19.** All of the following are structural polysaccharides EXCEPT:
(A) Cellulose
(B) Hemicellulose
(C) Pectin
(D) Glycogen

2009

**20.** Consider the following statements about polysaccharides:
I. Glycogen has more frequent branching than amylopectin.
II. Cellulose forms a planar, ribbon-like structure.
III. Pectin contains primarily xylose and arabinose residues.
Which of the above statements are correct?
(A) I and III
(B) I and II
(C) II and III
(D) I, II and III

2015

**21.** Which polysaccharide primarily contributes to the gel-forming ability in fruit jams and jellies?

(A) Amylose
(B) Cellulose
(C) Pectin
(D) Hemicellulose

2002

**22.** The structural organization of amylose is best described as:
(A) A left-handed single helix
(B) A highly branched network
(C) A double helix parallel structure
(D) A rigid β-pleated sheet

2008

**23.** The systematic nomenclature for linoleic acid, an essential fatty acid, is:
(A) 9,12,15-octadecatrienoic acid
(B) 9-octadecenoic acid
(C) 5,8,11,14-eicosatetraenoic acid
(D) 9,12-octadecadienoic acid

2007

**24.** Which of the following is considered an essential fatty acid for human nutrition typically derived from plants?
(A) Palmitic acid
(B) α-Linolenic acid
(C) Stearic acid
(D) Oleic acid

2012

**25.** Select the correct sequence of intermediates in the biosynthesis of fatty acids starting from acetyl-CoA.
(A) Acetyl-CoA → Malonyl-CoA → Acetoacetyl-ACP → β-Hydroxybutyryl-ACP
(B) Acetyl-CoA → Acetoacetyl-ACP → Malonyl-CoA → β-Hydroxybutyryl-ACP
(C) Malonyl-CoA → Acetyl-CoA → β-Hydroxybutyryl-ACP → Acetoacetyl-ACP
(D) Acetyl-CoA → β-Hydroxybutyryl-ACP → Malonyl-CoA → Acetoacetyl-ACP

2007

**26.** Which functional group primarily distinguishes unsaturated fatty acids from saturated fatty acids?
(A) Carboxyl group
(B) Hydroxyl group
(C) Methyl group
(D) Carbon-carbon double bond

2012

**27.** The first committed step in fatty acid biosynthesis is catalyzed by:
(A) Acetyl-CoA carboxylase
(B) Fatty acid synthase
(C) Malonyl-CoA decarboxylase
(D) Acyl-CoA dehydrogenase

2007

**28.** Identify the INCORRECT statement regarding triacylglycerols (TAGs).
(A) They are esters of glycerol and three fatty acids.
(B) They serve as major structural components of biological membranes.
(C) They are highly reduced and yield more energy per gram than carbohydrates.
(D) They are stored in specialized organelles called lipid droplets.

2016

**29.** Which biochemical pathway supplies the glycerol-3-phosphate backbone for triacylglycerol biosynthesis?
(A) Citric acid cycle
(B) Glycolysis
(C) Pentose phosphate pathway
(D) B-oxidation

2010

**30.** Consider the following statements about fatty acids:
I. Stearic acid is an 18-carbon saturated fatty acid.
II. Oleic acid contains a trans double bond at position 9.
III. Arachidonic acid has four cis double bonds.
IV. Essential fatty acids cannot be synthesized de novo by mammals.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II only
(C) III and IV
(D) I and IV

2024

**31.** The structural chemistry of naturally occurring unsaturated fatty acids usually features:
(A) Trans double bonds separated by a methylene group
(B) Conjugated double bonds throughout the chain
(C) Cis double bonds separated by a methylene group
(D) Alternating cis and trans double bonds

2006

**32.** What is the effect of increasing chain length on the physical properties of saturated fatty acids?

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(A) Decreased melting point and increased solubility in water
(B) Increased melting point and decreased solubility in water
(C) Decreased melting point and decreased solubility in water
(D) Increased melting point and increased solubility in water

2007

**33.** Which enzyme catalyzes the terminal step of triacylglycerol biosynthesis?
(A) Glycerol kinase
(B) Phosphatidic acid phosphatase
(C) Diacylglycerol acyltransferase (DGAT)
(D) Acyl-CoA synthetase

2010

**34.** A plant adapted to extreme cold would likely modify its membrane lipid composition by:
(A) Increasing the proportion of saturated fatty acids
(B) Increasing the proportion of unsaturated fatty acids
(C) Decreasing the total lipid content
(D) Increasing the length of fatty acid chains

2017

**35.** Arrange the following membrane lipids in order of their typical abundance in the thylakoid membranes of higher plants (highest to lowest):
I. Monogalactosyldiacylglycerol (MGDG)
II. Digalactosyldiacylglycerol (DGDG)
III. Phosphatidylglycerol (PG)
IV. Sulfoquinovosyldiacylglycerol (SQDG)
(A) I, II, IV, III
(B) II, I, III, IV
(C) I, II, III, IV
(D) III, I, II, IV

2010

**36.** Which lipid class is defined by a sphingosine backbone rather than glycerol?
(A) Sphingolipids
(B) Phosphoglycerides
(C) Galactolipids
(D) Triacylglycerols

2002

**37.** Phytosterols contribute to membrane fluidity primarily by:
(A) Covalently cross-linking the adjacent polar phospholipid head groups.
(B) Acting as rigid molecular plates that completely freeze the lipid membrane.
(C) Stabilizing fluidity by restricting motion while preventing close chain packing.
(D) Hydrolyzing phosphodiester bonds between phosphate and glycerol groups.

2002

**38.** Which of the following is the most abundant phospholipid found in the plasma membrane of higher plants?
(A) Phosphatidylcholine
(B) Phosphatidylglycerol
(C) Phosphatidylinositol
(D) Phosphatidylethanolamine

2017

**39.** Which of the following is an identifying feature of a glycolipid?
(A) A phosphate group attached to the polar head of the molecule.
(B) An esterified cholesterol molecule within the hydrophobic core region.
(C) A highly branched polyisoprenoid tail extending from the backbone.
(D) A carbohydrate moiety attached glycosidically to a lipid backbone.

2021

**40.** Consider the following statements about membrane lipids:
I. Phospholipids are amphipathic molecules.
II. Glycolipids in plants are predominantly found in plastid membranes.
III. Stigmasterol is a common phytosterol.
IV. Membrane fluidity increases with a higher ratio of saturated fatty acids.
V. Phytosterols share a tetracyclic core structure.
Which of the above statements are correct?
(A) I, II, III and V
(B) I, III, IV and V
(C) II, III, IV and V
(D) I, II, III and IV

2007

**41.** The biophysical property that allows lipid bilayers to spontaneously form in aqueous solutions is:
(A) The completely hydrophobic nature of lipids
(B) Covalent bonding between lipid tails
(C) Amphipathicity leading to the hydrophobic effect
(D) High specific heat capacity of water

2021

**42.** Which phytosterol serves as a direct precursor for the synthesis of brassinosteroids in plants?
(A) Sitosterol

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(B) Cholesterol
(C) Ergosterol
(D) Campesterol

2022

**43.** What is the fundamental consequence of a high phase transition temperature (Tm) in a lipid membrane?
(A) The membrane tends to be in a gel-like solid phase at ambient temperatures.
(B) The membrane is highly fluid at ambient temperatures.
(C) The membrane becomes freely permeable to ions.
(D) The membrane proteins diffuse more rapidly.

2017

**44.** Identify the correct pair associating a lipid with its location.
(A) MGDG : Plasma membrane
(B) Cardiolipin : Inner mitochondrial membrane
(C) Phosphatidylserine : Outer monolayer of plasma membrane
(D) Ergosterol : Animal cell membranes

2003

**45.** Which biophysical technique is most commonly used to measure the lateral diffusion of lipids in a membrane?
(A) X-ray crystallography
(B) Fluorescence Recovery After Photobleaching (FRAP)
(C) Mass spectrometry
(D) Nuclear Magnetic Resonance (NMR)

2001

**46.** At its isoelectric point (pI), an amino acid behaves as a:
(A) Zwitterion with zero net charge
(B) Purely positive ion
(C) Purely negative ion
(D) Neutral molecule with no charged groups

2005

**47.** Which of the following amino acids is classified as a non-protein amino acid commonly found in plants?
(A) Methionine
(B) Tryptophan
(C) Canavanine
(D) Histidine

2014

**48.** The titration curve of glycine shows two distinct buffering regions. These regions correspond to the pKa values of:
(A) Two α-carboxyl groups
(B) Two α-amino groups
(C) The α-carboxyl group and the side-chain R group
(D) The α-carboxyl group and the α-amino group

2023

**49.** Which of the following amino acids possesses a strongly basic side chain that is positively charged at physiological pH?
(A) Aspartate
(B) Serine
(C) Arginine
(D) Leucine

2004

**50.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The amino acid histidine is a crucial residue in the active sites of many enzymes.
Reason (R): The imidazole side chain of histidine has a pKa near physiological pH, allowing it to act as both an acid and a base.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**51.** Which classification correctly applies to the amino acid proline?
(A) Aromatic amino acid
(B) Acidic amino acid
(C) Sulfur-containing amino acid
(D) Imino acid

2024

**52.** What determines the specific physicochemical properties of an individual amino acid?
(A) The nature of the R-group (side chain)
(B) The α-amino group
(C) The α-carboxyl group
(D) The central α-carbon

2019

**53.** An amino acid with a pK1 of 2.34 and a pK2 of 9.60 will have an isoelectric point (pI) of approximately:
(A) 4.80
(B) 5.97
(C) 7.26

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(D) 11.94

2005

**54.** Identify the INCORRECT statement regarding non-protein amino acids in plants.
(A) They are major structural components of plant cell walls.
(B) They are never incorporated into proteins during translation.
(C) They often serve as defensive compounds against herbivores.
(D) GABA (gamma-aminobutyric acid) is an example of a non-protein amino acid.

2023

**55.** Which pair represents two sulfur-containing proteinaceous amino acids?
(A) Serine and Threonine
(B) Asparagine and Glutamine
(C) Phenylalanine and Tyrosine
(D) Methionine and Cysteine

2011

**56.** The absorption of ultraviolet light at 280 nm by proteins is primarily due to the presence of:
(A) Aliphatic amino acids
(B) Aromatic amino acids
(C) Basic amino acids
(D) Acidic amino acids

2006

**57.** The peptide bond in proteins has partial double-bond character primarily due to:
(A) Steric hindrance
(B) Hydrogen bonding
(C) Resonance
(D) Hydrophobic interactions

2011

**58.** Which level of protein structure describes the overall three-dimensional folding of a single polypeptide chain?
(A) Primary
(B) Secondary
(C) Quaternary
(D) Tertiary

2003

**59.** In an α-helix, the hydrogen bonds stabilize the structure by connecting:
(A) The carbonyl oxygen of residue i to the amide nitrogen of residue i+4
(B) The carbonyl oxygen of residue i to the amide nitrogen of residue i+3
(C) Adjacent R-groups
(D) Two separate polypeptide chains

2010

**60.** Match the protein structural features in List I with their descriptions in List II.
List I: (Structural Feature)
P. Primary structure
Q. Secondary structure
R. Tertiary structure
S. Quaternary structure
List II: (Description)
1. Assembly of multiple polypeptide subunits
2. Sequence of amino acids
3. Global folding stabilized by side-chain interactions
4. Local regular folding patterns like α-helices
(A) P-II, Q-IV, R-III, S-I
(B) P-II, Q-III, R-IV, S-I
(C) P-IV, Q-II, R-I, S-III
(D) P-I, Q-IV, R-III, S-II

2007

**61.** The Ramachandran plot displays the allowed values of which angles in a polypeptide backbone?
(A) Omega (peptide bond) and $\phi$
(B) $\phi$ (C-α-N) and $\psi$ (C-α-C)
(C) $\psi$ and Chi (side chain)
(D) $\phi$ and Omega

2011

**62.** Which amino acid is most likely to introduce a kink in an α-helix and is therefore termed a "helix breaker"?
(A) Proline
(B) Alanine
(C) Leucine
(D) Methionine

2014

**63.** A β-pleated sheet differs from an α-helix in that its hydrogen bonds:
(A) Exclusively intramolecular hydrogen bonds oriented parallel to the peptide chain.
(B) Hydrogen bonding interactions that only involve the amino acid side chains.
(C) Hydrogen bonds between adjacent strands perpendicular to the chain direction.
(D) Interactions that occur without any involvement of the peptide backbone atoms.

2015

**64.** Which non-covalent interaction is primarily responsible for burying hydrophobic amino acid residues in the interior of a globular protein?
(A) Hydrogen bonding
(B) Disulfide bridges
(C) Ionic bonding
(D) The hydrophobic effect

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2019

**65.** Identify the INCORRECT statement regarding the peptide bond geometry.
(A) It is planar.
(B) It is usually in the trans configuration.
(C) Free rotation is allowed around the C-N peptide bond.
(D) The C-N bond length is shorter than a typical C-N single bond.

2009

**66.** The quaternary structure of a protein refers specifically to:
(A) Its unique and specific primary amino acid sequence.
(B) The spatial arrangement of multiple subunits in a complex.
(C) The interaction of the protein with external prosthetic groups.
(D) The supersecondary structural motifs or domains present.

2005

**67.** Which of the following best explains why glycine occupies the largest area on a Ramachandran plot?
(A) Having a bulky side chain that allows for increased rotational freedom.
(B) Possessing a hydrogen atom side chain, minimizing steric hindrance.
(C) Frequently forming strong covalent disulfide bonds with other residues.
(D) Being localized exclusively in the flexible loop regions of proteins.

2022

**68.** A researcher identifies a structural motif composed of two α-helices joined by a short loop region, which frequently binds DNA. This motif is classified as:
(A) Supersecondary structure (motif)
(B) Quaternary structure
(C) Primary structure
(D) B-barrel

2004

**69.** The most common structural conformation of DNA under physiological conditions is:
(A) A-DNA
(B) Z-DNA
(C) B-DNA
(D) H-DNA

2004

**70.** Consider the following statements about nucleic acid structure:
I. B-DNA is a right-handed double helix.
II. Z-DNA is a left-handed helix with a zig-zag backbone.
III. A-DNA forms typically under conditions of high humidity.
Which of the above statements are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2024

**71.** In DNA base pairing, the predominant tautomeric forms of the nitrogenous bases are:
(A) Enol and imino forms
(B) Keto and imino forms
(C) Enol and amino forms
(D) Keto and amino forms

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2012

**72.** Which term describes the temperature at which 50% of double-stranded DNA is denatured into single strands?
(A) Annealing temperature (Ta)
(B) Melting temperature (Tm)
(C) Denaturation optimum
(D) Renaturation temperature

2006

**73.** An increase in the GC content of a DNA molecule will lead to:
(A) A decrease in its melting temperature (Tm)
(B) No change in its melting temperature
(C) An increase in its melting temperature (Tm)
(D) Immediate conversion to Z-DNA

2022

**74.** Which stabilizing force contributes most significantly to the thermodynamic stability of the DNA double helix?
(A) Highly specific hydrogen bonds formed between individual base pairs.
(B) Covalent phosphodiester bonds within the sugar-phosphate backbone.
(C) Electrostatic ionic interactions between adjacent negatively charged phosphate groups.
(D) Base-stacking interactions involving Van der Waals and hydrophobic forces.

2017

**75.** Identify the INCORRECT pair regarding nucleic acids.
(A) Uracil : Found in DNA
(B) Adenine : Purine
(C) Cytosine : Pyrimidine

(D) Thymine : Contains a methyl group

2025

**76.** When double-stranded DNA undergoes denaturation, its ultraviolet absorbance at 260 nm increases. This phenomenon is known as:
(A) The hypochromic effect
(B) Bathochromic shift
(C) The hyperchromic effect
(D) Fluorescence resonance

2010

**77.** The glycosidic linkage in a nucleotide connects the pentose sugar to the nitrogenous base at which position of the sugar?
(A) C-2'
(B) C-1'
(C) C-3'
(D) C-5'

2001

**78.** What structural feature uniquely distinguishes RNA from DNA?
(A) The 2'-hydroxyl group on the ribose pentose ring.
(B) The 3'-hydroxyl group on the sugar moiety.
(C) The repeating covalent sugar-phosphodiester backbone.
(D) The right-handed nature of the helical structure.

2011

**79.** The conversion of a normal amino tautomer of adenine to its imino form can lead to:
(A) Spontaneous DNA breakage
(B) Immediate helix unwinding
(C) A transition mutation during replication
(D) Cross-linking of DNA strands

2010

**80.** Consider the following statements about glycolysis (EMP pathway):
I. It occurs in the cytoplasm.
II. Phosphofructokinase is the key regulatory enzyme.
III. It generates a net of 4 ATP per glucose.
IV. Pyruvate kinase catalyzes an irreversible substrate-level phosphorylation.
Which of the above statements are correct?
(A) I, II and III
(B) I, II and IV
(C) II, III and IV
(D) I, III and IV

2023

**81.** In the first step of glycolysis, hexokinase catalyzes the conversion of glucose to glucose-6-phosphate. This reaction is an example of:
(A) Isomerization
(B) Substrate-level phosphorylation
(C) Phosphorylation requiring ATP
(D) Oxidative phosphorylation

2023

**82.** Which glycolytic enzyme is strongly inhibited by high concentrations of ATP and citrate, serving as a primary metabolic control point?
(A) Hexokinase
(B) Phosphofructokinase-1 (PFK-1)
(C) Aldolase
(D) Enolase

2019

**83.** The cleavage of fructose-1,6-bisphosphate yields which two triose phosphates?
(A) 3-Phosphoglycerate and Phosphoenolpyruvate
(B) Glycerol-3-phosphate and Pyruvate
(C) 1,3-Bisphosphoglycerate and Dihydroxyacetone phosphate
(D) Dihydroxyacetone phosphate and Glyceraldehyde-3-phosphate

2011

**84.** Which step in the EMP pathway generates NADH?
(A) Conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate
(B) Conversion of glucose-6-phosphate to fructose-6-phosphate
(C) Conversion of 2-phosphoglycerate to phosphoenolpyruvate
(D) Conversion of phosphoenolpyruvate to pyruvate

2004

**85.** During anaerobic fermentation in plants (e.g., in flooded roots), pyruvate is primarily converted to:
(A) Lactic acid
(B) Acetyl-CoA
(C) Ethanol and $CO_2$
(D) Oxaloacetate

2021

**86.** The energetic yield of glycolysis from one molecule of glucose under aerobic conditions strictly in terms of direct ATP net production is:
(A) 2 ATP
(B) 4 ATP

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(C) 32 ATP
(D) 36 ATP

2023

**87.** Identify the enzyme that catalyzes a reversible reaction in glycolysis.
(A) Hexokinase
(B) Phosphofructokinase
(C) Pyruvate kinase
(D) Phosphoglycerate kinase

2003

**88.** Fluoride is a well-known inhibitor of glycolysis. Which specific enzyme does it inhibit?
(A) Hexokinase
(B) Glyceraldehyde-3-phosphate dehydrogenase
(C) Enolase
(D) Pyruvate kinase

2005

**89.** Which statement INCORRECTLY characterizes the EMP pathway?
(A) It operates anaerobically.
(B) It involves two investment ATPs.
(C) It occurs universally in almost all living cells.
(D) It completely oxidizes glucose to $CO_2$ and $H_2O$.

2004

**90.** Consider the following statements about anaerobic fermentation:
I. It regenerates NAD+ for glycolysis to continue.
II. Lactate dehydrogenase is required for ethanol fermentation.
III. Alcohol dehydrogenase catalyzes the reduction of acetaldehyde to ethanol.
IV. It produces more ATP than oxidative phosphorylation.
V. Pyruvate decarboxylase releases $CO_2$ during alcoholic fermentation.
Which of the above statements are correct?
(A) I, II, and III
(B) I, III, and V
(C) II, IV, and V
(D) I, IV, and V

2018

**91.** The primary metabolic significance of the oxidative phase of the Pentose Phosphate Pathway (HMP shunt) is to generate:
(A) NADPH and Ribose-5-phosphate
(B) ATP and NADH
(C) FADH2 and Ribulose-5-phosphate
(D) Pyruvate and ATP

2015

**92.** Which enzyme catalyzes the committed and rate-limiting step of the Pentose Phosphate Pathway?
(A) Transketolase
(B) Glucose-6-phosphate dehydrogenase
(C) 6-Phosphogluconate dehydrogenase
(D) Transaldolase

2014

**93.** In the non-oxidative phase of the HMP shunt, which coenzyme is strictly required by the enzyme transketolase?
(A) NADP+
(B) Thiamine pyrophosphate (TPP)
(C) Pyridoxal phosphate (PLP)
(D) FAD

2024

**94.** The NADPH generated by the Pentose Phosphate Pathway is predominantly used for:
(A) Reductive biosynthetic reactions and antioxidant defense
(B) Driving the mitochondrial electron transport chain
(C) Generating a proton gradient across the thylakoid membrane
(D) Hydrolyzing ATP in the cytosol

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2007

**95.** Identify the correct statement regarding the HMP shunt.
(A) It operates exclusively in the mitochondria.
(B) It does not share any intermediates with glycolysis.
(C) It is highly active in tissues undergoing extensive fatty acid synthesis.
(D) The oxidative phase is highly reversible.

2013

**96.** Which 4-carbon sugar intermediate is uniquely produced during the non-oxidative phase of the Pentose Phosphate Pathway?
(A) Xylulose-5-phosphate
(B) Sedoheptulose-7-phosphate
(C) Ribose-5-phosphate
(D) Erythrose-4-phosphate

2008

**97.** The conversion of ribulose-5-phosphate to xylulose-5-phosphate is catalyzed by:
(A) An isomerase
(B) A kinase
(C) An epimerase
(D) A mutase

2025

**98.** Transaldolase transfers a carbon fragment of what size between sugar molecules?
(A) 1-carbon fragment
(B) 2-carbon fragment
(C) 3-carbon fragment
(D) 4-carbon fragment

2016

**99.** The generation of reducing equivalents (NADPH) in the PPP occurs during the oxidation of:
(A) Fructose-6-phosphate and Glyceraldehyde-3-phosphate
(B) Ribose-5-phosphate and Xylulose-5-phosphate
(C) Erythrose-4-phosphate and Sedoheptulose-7-phosphate
(D) Glucose-6-phosphate and 6-phosphogluconate

2006

**100.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The Pentose Phosphate Pathway is critical for nucleotide biosynthesis.
Reason (R): The oxidative phase of the pathway provides the ribose-5-phosphate required for the synthesis of purine and pyrimidine nucleotides.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2004

**101.** Which condition would most likely allosterically activate Glucose-6-phosphate dehydrogenase?
(A) High levels of ATP
(B) High levels of NADPH
(C) High levels of NADP+
(D) Low levels of NADP+

2014

**102.** Which of the following is an INCORRECT statement about the Pentose Phosphate Pathway?
(A) It consumes ATP during the non-oxidative phase.
(B) It generates $CO_2$ during the oxidative phase.
(C) It can be conceptually divided into oxidative and non-oxidative phases.
(D) Sedoheptulose-7-phosphate is a 7-carbon intermediate.

2022

**103.** The Citric Acid Cycle (TCA cycle) is initiated by the condensation of acetyl-CoA with:
(A) Pyruvate
(B) Citrate
(C) Malate
(D) Oxaloacetate

2025

**104.** Which multi-enzyme complex serves as the critical link between glycolysis and the TCA cycle?
(A) A-ketoglutarate dehydrogenase complex
(B) Pyruvate dehydrogenase complex
(C) Succinate dehydrogenase complex
(D) Malate dehydrogenase

2015

**105.** The amphibolic nature of the TCA cycle refers to its ability to:
(A) Operate under both aerobic and anaerobic conditions
(B) Generate both ATP and GTP
(C) Function in both catabolic and anabolic pathways
(D) Occur in both the cytosol and mitochondria

2019

**106.** Which enzyme of the TCA cycle is uniquely embedded in the inner mitochondrial membrane?
(A) Citrate synthase
(B) Isocitrate dehydrogenase
(C) Fumarase
(D) Succinate dehydrogenase

2013

**107.** During one complete turn of the TCA cycle starting from one acetyl-CoA, how many molecules of NADH are generated?
(A) 1
(B) 2
(C) 3
(D) 4

2015

**108.** The conversion of succinyl-CoA to succinate is coupled with the substrate-level phosphorylation of GDP to GTP (or ADP to ATP in some organisms). This reaction is catalyzed by:
(A) Succinate dehydrogenase
(B) Succinyl-CoA synthetase (thiokinase)
(C) A-ketoglutarate dehydrogenase
(D) Fumarase

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2005

**109.** Which intermediate of the TCA cycle acts as a precursor for porphyrin and chlorophyll biosynthesis?
(A) Oxaloacetate
(B) Succinyl-CoA
(C) Citrate
(D) A-ketoglutarate

2007

**110.** Match the TCA cycle enzymes in List I with their corresponding reaction products in List II.
List I: (Enzyme)
P. Citrate synthase
Q. Aconitase
R. Isocitrate dehydrogenase
S. Fumarase
List II: (Product)
1. Isocitrate
2. Malate
3. Citrate
4. A-ketoglutarate
(A) P-III, Q-I, R-IV, S-II
(B) P-III, Q-IV, R-I, S-II
(C) P-I, Q-III, R-II, S-IV
(D) P-II, Q-I, R-IV, S-III

2011

**111.** Which inhibitor blocks the TCA cycle by acting as a structural analog and competitive inhibitor of succinate dehydrogenase?
(A) Fluorocitrate
(B) Arsenite
(C) Malonate
(D) Cyanide

2019

**112.** The oxidative decarboxylation steps in the TCA cycle release $CO_2$. These steps are catalyzed by:
(A) Citrate synthase and aconitase
(B) Succinate dehydrogenase and malate dehydrogenase
(C) Fumarase and malate dehydrogenase
(D) Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase

2022

**113.** Fluoroacetate is toxic because it is converted into fluorocitrate, which is a potent inhibitor of:
(A) Pyruvate dehydrogenase
(B) Succinyl-CoA synthetase
(C) Aconitase
(D) Citrate synthase

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2013

**114.** The regulation of the Pyruvate Dehydrogenase Complex in plants is heavily controlled by reversible covalent modification via:
(A) Adenylation/Deadenylation
(B) Phosphorylation/Dephosphorylation
(C) Acetylation/Deacetylation
(D) Methylation/Demethylation

2006

**115.** Gluconeogenesis is the metabolic pathway responsible for:
(A) The breakdown of glycogen to glucose
(B) The conversion of glucose to fatty acids
(C) The oxidation of glucose to yield ATP
(D) The synthesis of glucose from non-carbohydrate precursors

2011

**116.** In germinating oilseeds, the spatial coordination of gluconeogenesis begins with the breakdown of fatty acids in which organelle?
(A) Glyoxysome
(B) Mitochondria
(C) Chloroplast
(D) Peroxisome

2022

**117.** Which two unique enzymes are present in the glyoxylate cycle that bypass the decarboxylation steps of the TCA cycle?
(A) Citrate synthase and Aconitase
(B) Malate dehydrogenase and Fumarase
(C) Isocitrate lyase and Malate synthase
(D) Phosphoenolpyruvate carboxykinase and Pyruvate carboxylase

2007

**118.** The net result of one turn of the glyoxylate cycle is the conversion of two molecules of acetyl-CoA into one molecule of:
(A) Succinate
(B) Citrate
(C) Oxaloacetate
(D) Glyoxylate

2022

**119.** Which enzyme in gluconeogenesis bypasses the irreversible pyruvate kinase reaction of glycolysis?
(A) Fructose-1,6-bisphosphatase enzyme complex.
(B) Glucose-6-phosphatase regulatory enzyme.
(C) Hexokinase-mediated phosphorylation reaction.
(D) Pyruvate carboxylase and PEP

carboxykinase.

2018

**120.** Consider the following statements about gluconeogenesis and the glyoxylate cycle:
I. The glyoxylate cycle occurs primarily in the glyoxysomes of germinating seeds.
II. Succinate produced in the glyoxysome is transported to the mitochondria.
III. Gluconeogenesis does not consume ATP.
Which of the above statements are correct?
(A) I and III
(B) I and II
(C) II and III
(D) I, II and III

2008

**121.** During the conversion of storage lipids to carbohydrates in germinating seeds, malate is transported from the mitochondria to the cytosol where it is oxidized to:
(A) Pyruvate
(B) Phosphoenolpyruvate
(C) Oxaloacetate
(D) Fumarate

2007

**122.** Which enzyme catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate in gluconeogenesis?
(A) Phosphofructokinase
(B) Aldolase
(C) Hexokinase
(D) Fructose-1,6-bisphosphatase

2002

**123.** The glyoxylate cycle prevents the loss of carbon as $CO_2$. This allows plants and some microbes to survive on:
(A) Acetate or fatty acids as the sole carbon source.
(B) Sunlight and water via the photosynthetic pathway.
(C) Diets based exclusively on high protein intake.
(D) Starch deposits found within the amyloplast organelle.

2009

**124.** Which is the correct biological significance of the glyoxylate cycle in plants?
(A) It generates substantial amounts of ATP for immediate cellular energy.
(B) Converting stored fats to carbohydrates for fueling seedling growth.
(C) It acts primarily as a localized antioxidant defense mechanism.
(D) It replaces the TCA cycle in the leaves of adult plant species.

2017

**125.** The conversion of oxaloacetate to phosphoenolpyruvate (PEP) in gluconeogenesis utilizes which high-energy phosphate donor?
(A) ATP
(B) GTP or ATP depending on the organism/ enzyme variant
(C) UTP
(D) CTP

2011

**126.** The mitochondrial Electron Transport Chain (ETC) transfers electrons from NADH and FADH2 to:
(A) Molecular oxygen
(B) Carbon dioxide
(C) Water
(D) Cytochrome c

2010

**127.** Which complex of the ETC is responsible for oxidizing NADH?
(A) Complex II (Succinate dehydrogenase)
(B) Complex III (Cytochrome bc1 complex)
(C) Complex I (NADH dehydrogenase)
(D) Complex IV (Cytochrome c oxidase)

2019

**128.** Coenzyme Q (Ubiquinone) functions in the ETC as a:
(A) Fixed integral membrane protein
(B) Peripheral water-soluble protein
(C) Terminal electron acceptor
(D) Mobile lipid-soluble electron carrier

2022

**129.** The alternative oxidase (AOX) pathway in plant mitochondria branches off the main ETC at which component?
(A) Complex I
(B) Complex III
(C) Ubiquinone pool
(D) Cytochrome c

2017

**130.** Consider the following statements about the plant mitochondrial ETC:
I. Complex IV reduces $O_2$ to $H_2O$.
II. AOX is inhibited by cyanide.
III. Complex II does not pump protons.
IV. The AOX pathway dissipates energy as heat.
Which of the above statements are correct?

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(A) I, II and III
(B) I, III and IV
(C) II, III and IV
(D) I, II, III and IV

2021

**131.** Which specific inhibitor effectively blocks electron transfer at Complex IV (Cytochrome c oxidase)?
(A) Rotenone
(B) Antimycin A
(C) Oligomycin
(D) Cyanide

2014

**132.** The physiological role of the Alternative Oxidase (AOX) pathway in thermogenic plants (e.g., Arum lilies) is to:
(A) Generating heat to volatilize scents to attract pollinators.
(B) Maximizing ATP production through the electron transport chain.
(C) Synthesizing massive quantities of water for metabolic processes.
(D) Preventing the excessive intake of oxygen into the mitochondria.

2011

**133.** Which component of the ETC is a mobile, water-soluble peripheral membrane protein located in the intermembrane space?
(A) Ubiquinone
(B) Iron-sulfur clusters
(C) Cytochrome c
(D) FMN

2014

**134.** Rotenone is a specific, potent inhibitor of which multi-protein complex in the ETC?
(A) Complex I
(B) Complex II
(C) Complex III
(D) Complex IV

2001

**135.** The transfer of electrons through Complexes I, III, and IV is coupled to the transport of what across the inner mitochondrial membrane?
(A) Electrons
(B) Sodium ions
(C) Potassium ions
(D) Protons

2019

**136.** Which statement INCORRECTLY describes the Alternative Oxidase (AOX) pathway?
(A) It provides a mechanism to prevent over-reduction of the ubiquinone pool.
(B) It is uncoupled from proton pumping at Complexes III and IV.
(C) It helps mitigate the production of reactive oxygen species (ROS).
(D) It is highly sensitive and completely inhibited by potassium cyanide.

2023

**137.** The mechanism linking electron transport to ATP synthesis is best described by the:
(A) Lock and key hypothesis
(B) Induced fit model
(C) Chemiosmotic theory
(D) Substrate-level phosphorylation mechanism

2017

**138.** According to the Chemiosmotic theory, the energy derived from electron transport is temporarily stored as:
(A) High-energy phosphate bonds in various metabolic intermediates.
(B) Thermal energy dissipated as heat within the mitochondrial matrix.
(C) Reducing power stored in the form of mitochondrial NADH molecules.
(D) Electrochemical proton gradient known as the proton-motive force.

2008

**139.** The $F_1F_0$-ATP synthase utilizes the flow of protons back into the mitochondrial matrix to drive ATP synthesis via:
(A) Rotational catalysis
(B) Linear electron flow
(C) Substrate-level phosphorylation
(D) Covalent modification

2019

**140.** Consider the following statements about $F_1F_0$-ATP synthase:
I. The $F_0$ portion is embedded in the inner mitochondrial membrane.
II. The $F_1$ portion contains the catalytic sites for ATP synthesis.
III. Rotation of the gamma subunit causes conformational changes in the $\alpha$ and $\beta$ subunits.
IV. Oligomycin inhibits ATP synthase by blocking the proton channel of $F_0$.
V. It can only synthesize ATP and cannot hydrolyze it under any condition.
Which of the above statements are correct?
(A) I, II, III and IV

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(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2011

**141.** Who proposed the Chemiosmotic hypothesis for oxidative phosphorylation?
(A) Hans Krebs
(B) Peter Mitchell
(C) Melvin Calvin
(D) Otto Warburg

2003

**142.** Which chemical acts as an uncoupler of oxidative phosphorylation by carrying protons across the inner mitochondrial membrane, thus dissipating the gradient?
(A) 2,4-Dinitrophenol (DNP).
(B) Rotenone (Complex I inhibitor).
(C) Cyanide (Complex IV inhibitor).
(D) Oligomycin (ATP synthase inhibitor).

2021

**143.** In rotational catalysis by ATP synthase, the three catalytic β subunits cycle through three conformational states designated as:
(A) Active, Inactive, Blocked
(B) Bound, Unbound, Transition
(C) Open, Loose, Tight (O, L, T)
(D) A, B, Gamma

2014

**144.** Substrate-level phosphorylation differs from oxidative phosphorylation in that it:
(A) Occurs exclusively within the mitochondrial matrix compartment.
(B) Requiring the presence of a strong electrochemical proton-motive force.
(C) Relying entirely on molecular oxygen as the final electron acceptor.
(D) Transferring a phosphoryl group from a high-energy substrate to ADP.

2003

**145.** An uncoupling agent like DNP added to actively respiring mitochondria will cause:
(A) A halt in oxygen consumption
(B) An increase in ATP synthesis
(C) Continued oxygen consumption but halted ATP synthesis
(D) Immediate destruction of the ATP synthase complex

2013

**146.** Which part of the $F_1F_0$-ATP synthase complex forms the rotating ring that conducts protons?
(A) The $\alpha_3\beta_3$ hexamer assembly.
(B) The c-ring of the $F_0$ sector.
(C) The delta subunit of the stalk.
(D) The membrane-bound a-subunit protein.

2011

**147.** Under standard conditions, the theoretical yield of ATP per molecule of NADH oxidized via the mammalian/plant ETC is approximately:
(A) 1.5
(B) 2.5
(C) 3.5
(D) 4.0

2010

**148.** Identify the INCORRECT statement concerning oxidative phosphorylation.
(A) Protons are pumped from the intermembrane space into the matrix by the ETC.
(B) It is strictly dependent on the integrity of the inner mitochondrial membrane.
(C) It is driven by the proton-motive force consisting of Δ-pH and Δ-ψ.
(D) ATP synthesis occurs in the matrix compartment.

2006

**149.** Vitamin C (Ascorbic acid) serves biochemically as a:
(A) Coenzyme for various metabolic decarboxylases.
(B) Structural component of essential cell membranes.
(C) Antioxidant cofactor involved in collagen synthesis.
(D) Precursor for various light-sensing visual pigments.

2012

**150.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Night blindness is a common symptom of Vitamin A deficiency.
Reason (R): Retinal, a derivative of Vitamin A, is a crucial component of the visual pigment rhodopsin.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2001

**151.** Which fat-soluble vitamin primarily functions as a lipid-soluble antioxidant in cellular membranes, protecting against lipid peroxidation?
(A) Vitamin A
(B) Vitamin D
(C) Vitamin K
(D) Vitamin E (Tocopherol)

2001

**152.** The coenzyme form of Thiamine (Vitamin B1) is essential for which type of biochemical reaction?
(A) Transamination
(B) Oxidative decarboxylation
(C) Methyl group transfer
(D) Carboxylation

2012

**153.** A deficiency in Vitamin K severely impacts blood coagulation because it is required as a cofactor for the:
(A) Hydroxylation of proline residues.
(B) Phosphorylation of fibrinogen molecules.
(C) Gamma-carboxylation of clotting factors.
(D) Proteolytic cleavage of prothrombin proteins.

2005

**154.** Pellagra, characterized by dermatitis, diarrhea, and dementia, is caused by a severe deficiency of:
(A) Riboflavin (B2)
(B) Pyridoxine (B6)
(C) Cobalamin (B12)
(D) Niacin (B3)

2022

**155.** Which vitamin functions as a precursor for the biosynthesis of the electron carriers NAD+ and NADP+?
(A) Niacin
(B) Thiamine
(C) Riboflavin
(D) Pantothenic acid

2001

**156.** The active coenzyme form of Vitamin B6 (Pyridoxine) is primarily involved in:
(A) Fatty acid oxidation
(B) Glycogen synthesis
(C) DNA replication
(D) Amino acid metabolism (e.g., transamination)

2023

**157.** Which vitamin relies on UV radiation (sunlight) for its endogenous synthesis from a sterol precursor in humans and animals?
(A) Vitamin C
(B) Vitamin D
(C) Vitamin E
(D) Vitamin K

2009

**158.** Pantothenic acid (Vitamin B5) is a vital structural component of which ubiquitous metabolic molecule?
(A) Coenzyme A
(B) Flavin Adenine Dinucleotide (FAD)
(C) Tetrahydrofolate
(D) S-adenosylmethionine (SAM)

2014

**159.** Identify the INCORRECT statement regarding vitamins and their deficiency symptoms.
(A) Vitamin C : Scurvy
(B) Vitamin D : Rickets
(C) Vitamin B12 : Night blindness
(D) Vitamin B1 : Beriberi

2003

**160.** Match the plant hormones in List I with their primary biosynthetic precursors in List II.
List I: (Hormone)
P. Auxin (IAA)
Q. Gibberellin (GA)
R. Cytokinin
S. Ethylene
List II: (Precursor)
1. Methionine
2. Tryptophan
3. GGPP (Diterpene pathway)
4. Adenine / Isopentenyl pyrophosphate
(A) P-II, Q-III, R-IV, S-I
(B) P-III, Q-II, R-I, S-IV
(C) P-II, Q-IV, R-III, S-I
(D) P-I, Q-III, R-IV, S-II

2009

**161.** The biosynthesis of Gibberellins fundamentally occurs via the:
(A) Shikimate pathway exclusively
(B) Lipoxygenase pathway
(C) Mevalonate and MEP (terpenoid) pathways
(D) Phenylpropanoid pathway

2003

**162.** Auxin is characterized by its unique mode of polar transport. Which class of efflux carrier proteins mediates this directional transport?
(A) ABC transporters
(B) PIN proteins
(C) Aquaporins
(D) AUX1 importers

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2018

**163.** The signal transduction mechanism of Auxin involves the degradation of repressor proteins. These specific repressor proteins are called:
(A) DELLA proteins
(B) JAZ proteins
(C) ARR proteins
(D) Aux/IAA proteins

2007

**164.** Which cellular receptor directly binds Auxin to initiate its signaling cascade?
(A) TIR1
(B) GID1
(C) ETR1
(D) CRE1

2004

**165.** Cytokinins primarily promote which physiological process in plants?
(A) Prompt stomatal closure.
(B) Rapid stem elongation.
(C) Promoting cell division.
(D) Accelerated fruit ripening.

2002

**166.** Gibberellin signal transduction facilitates seed germination primarily by inducing the synthesis of:
(A) A-amylase
(B) Cellulase
(C) Lipase
(D) Protease

2014

**167.** The receptor for Gibberellins in plant cells is known as:
(A) TIR1
(B) BRI1
(C) PYR1
(D) GID1

2001

**168.** Which pathway provides the aromatic precursor tryptophan required for the de novo biosynthesis of Indole-3-acetic acid (IAA)?
(A) Pentose phosphate pathway
(B) Shikimate pathway
(C) MEP pathway
(D) Glyoxylate cycle

2021

**169.** In Cytokinin signaling, the mechanism relies on a modified version of a bacterial regulatory system known as the:
(A) G-protein coupled cascade
(B) Kinase cascade (MAPK)
(C) Two-component phosphorelay system
(D) Ubiquitin-proteasome system

2017

**170.** Which of the following is considered the primary naturally occurring active Cytokinin in higher plants?
(A) Kinetin
(B) Benzyladenine
(C) Thidiazuron
(D) Zeatin

2025

**171.** The biosynthesis of Abscisic Acid (ABA) in plants originates directly from the cleavage of:
(A) A C40 carotenoid precursor (e.g., zeaxanthin)
(B) A C20 diterpene
(C) A C15 sesquiterpene generated directly from FPP
(D) Tryptophan

2004

**172.** Ethylene biosynthesis utilizes the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). The enzyme converting ACC to ethylene is:
(A) ACC synthase (ACS)
(B) ACC oxidase (ACO)
(C) S-adenosylmethionine synthetase
(D) Ethylene forming enzyme complex

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2011

**173.** Jasmonates are lipid-derived phytohormones synthesized from:
(A) Oleic acid via β-oxidation
(B) Linolenic acid via the lipoxygenase (LOX) pathway
(C) Stearic acid via elongation
(D) Palmitic acid via desaturation

2012

**174.** Salicylic acid biosynthesis in plants predominantly occurs via the:
(A) Isochorismate pathway and/or Phenylalanine ammonia-lyase (PAL) pathway
(B) Mevalonate pathway
(C) MEP pathway
(D) Lipoxygenase pathway

2011

**175.** Select the correct sequence of intermediate compounds in the synthesis of ethylene from its primary amino acid precursor.
(A) Methionine → ACC → SAM → Ethylene
(B) Methionine → SAM → ACC → Ethylene
(C) SAM → Methionine → ACC → Ethylene
(D) ACC → Methionine → SAM → Ethylene

2002

**176.** Brassinosteroids function biologically to promote:
(A) Seed dormancy and stomatal closure
(B) Abscission and fruit ripening
(C) Systemic acquired resistance (SAR) against pathogens
(D) Cell expansion, elongation, and resistance to environmental stress

2022

**177.** The mode of action of Abscisic Acid (ABA) during drought stress primarily involves:
(A) Promoting rapid root elongation
(B) Increasing leaf surface area
(C) Inducing stomatal closure to minimize transpirational water loss
(D) Inducing α-amylase in guard cells

2009

**178.** Which phytohormone is critically required for establishing Systemic Acquired Resistance (SAR) after a pathogen attack?
(A) Ethylene
(B) Salicylic Acid
(C) Jasmonic Acid
(D) Brassinosteroids

2018

**179.** Ethylene perception in plants involves a family of receptors (e.g., ETR1) located primarily on the membrane of the:
(A) Plasma membrane
(B) Tonoplast
(C) Mitochondria
(D) Endoplasmic reticulum

2013

**180.** The JAZ proteins act as repressors in the signal transduction pathway of which hormone?
(A) Jasmonates
(B) Auxin
(C) Salicylic acid
(D) Abscisic acid

2005

**181.** Brassinosteroid signaling involves the receptor kinase BRI1 and leads to the dephosphorylation and activation of transcription factors known as:
(A) $ARF_1$ and ARF2
(B) DELLA proteins
(C) BZR1 and BES1
(D) MYC2

2024

**182.** Identify the INCORRECT statement regarding plant stress hormones.
(A) Ethylene acts exclusively as an inhibitor of senescence.
(B) ABA signaling requires the PYR/PYL/RCAR family of receptors.
(C) Jasmonic acid is primarily involved in defense against necrotrophic pathogens and herbivores.
(D) Salicylic acid can induce the synthesis of pathogenesis-related (PR) proteins.

2017

**183.** The enzymatic function of Superoxide Dismutase (SOD) is to catalyze the dismutation of superoxide radicals into:
(A) Oxygen and water
(B) Singlet oxygen and water
(C) Hydroxyl radicals and water
(D) Hydrogen peroxide and oxygen

2015

**184.** Which metal is NOT commonly found as a cofactor in plant Superoxide Dismutase (SOD) isoforms?
(A) Copper/Zinc
(B) Molybdenum
(C) Manganese
(D) Iron

2017

**185.** Arrange the enzymatic steps of the Halliwell-Asada (Ascorbate-Glutathione) cycle in the correct operational sequence for detoxifying $H_2O_2$.
(A) $H_2O_2$ is reduced by Ascorbate Peroxidase → Monodehydroascorbate is regenerated → Glutathione is oxidized → Glutathione Reductase reduces GSSG
(B) Glutathione Reductase reduces GSSG → $H_2O_2$ is reduced by Ascorbate Peroxidase → Monodehydroascorbate is regenerated → Glutathione is oxidized
(C) Monodehydroascorbate is regenerated → $H_2O_2$ is reduced by Ascorbate Peroxidase → Glutathione is oxidized → Glutathione Reductase reduces GSSG
(D) $H_2O_2$ is reduced by Ascorbate Peroxidase → Glutathione is oxidized → Glutathione Reductase reduces GSSG → Monodehydroascorbate is regenerated

2010

**186.** Catalase (CAT) predominantly localizes to which subcellular organelle to scavenge hydrogen peroxide?
(A) Chloroplast
(B) Mitochondrion

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(C) Vacuole
(D) Peroxisome

2008

**187.** Guaiacol Peroxidase (GPX) utilizes hydrogen peroxide to oxidize a variety of substrates, primarily functioning in:
(A) Lignin biosynthesis and cell wall cross-linking
(B) Photosynthetic electron transport
(C) Nitrogen fixation
(D) The synthesis of gibberellins

2024

**188.** In the Ascorbate-Glutathione cycle, Ascorbate Peroxidase (APX) utilizes ascorbate as a specific electron donor to reduce:
(A) Superoxide
(B) Singlet oxygen
(C) Hydrogen peroxide
(D) Hydroxyl radicals

2011

**189.** The enzyme Glutathione Reductase strictly requires which coenzyme to reduce oxidized glutathione (GSSG) back to GSH?
(A) NADPH
(B) NADH
(C) FADH2
(D) ATP

2018

**190.** The primary biological necessity for antioxidant defense systems in plants is to:
(A) Preventing cellular damage from metabolic and stress-induced ROS.
(B) Synthesizing carbohydrates via the Calvin cycle during photosynthesis.
(C) Increasing the rate of photorespiration under low carbon conditions.
(D) Storing toxic heavy metals safely within the plant's central vacuole.

2018

**191.** Dehydroascorbate reductase (DHAR) utilizes which molecule as an electron donor to regenerate ascorbic acid?
(A) NADPH
(B) Reduced ferredoxin
(C) Reduced glutathione (GSH)
(D) NADH

2001

**192.** Which of the following ROS is the most reactive and toxic, for which plants possess no specific scavenging enzyme?
(A) Superoxide radical ($O_2^{\cdot -}$)
(B) Hydrogen peroxide ($H_2O_2$)
(C) Singlet oxygen ($^1O_2$)
(D) Hydroxyl radical ($^{\cdot}OH$)

2014

**193.** Identify the INCORRECT statement regarding the Ascorbate-Glutathione pathway.
(A) It is exclusively localized in the cytosol.
(B) It involves the cyclic oxidation and reduction of ascorbate and glutathione.
(C) It relies on NADPH to maintain the pool of reduced antioxidants.
(D) It is heavily involved in stress tolerance in chloroplasts.

2025

**194.** Non-enzymatic plant antioxidants like carotenoids primarily protect the photosynthetic apparatus by:
(A) Absorbing infrared radiation
(B) Quenching triplet chlorophyll and singlet oxygen
(C) Chelating heavy metals
(D) Synthesizing ATP

2006

**195.** Tocopherols (Vitamin E) are lipophilic antioxidants predominantly synthesized and localized in the:
(A) Cytosol
(B) Vacuole
(C) Endoplasmic reticulum
(D) Plastids (chloroplast membranes)

2016

**196.** Flavonoids function as potent antioxidants largely due to their structural chemistry, which features:
(A) Polyhydroxylated phenolic rings
(B) Multiple double bonds in a linear carbon chain
(C) A highly saturated steroid backbone
(D) A porphyrin ring coordinating magnesium

2014

**197.** Ascorbic acid functions non-enzymatically in plants by directly reacting with and neutralizing:
(A) Cytosolic calcium ions.
(B) Free glucose molecules.
(C) Superoxide and singlet oxygen.
(D) Dissolved nitrate ions.

2013

**198.** B-carotene is an example of which class of non-enzymatic antioxidants?

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(A) Carotenoids
(B) Flavonoids
(C) Tocopherols
(D) Alkaloids

2004

**199.** The antioxidant capacity of tocopherols lies in their ability to terminate lipid peroxidation chain reactions. The reactive portion of the tocopherol molecule is the:
(A) Phytyl tail
(B) Carboxyl group
(C) Glycosidic linkage
(D) Chromanol ring

2004

**200.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Anthocyanins often accumulate in plant leaves under high-light or cold stress conditions.
Reason (R): Anthocyanins serve as photoprotectants and ROS scavengers in the vacuole.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**201.** Which class of flavonoids predominantly absorbs UV-B radiation, thereby protecting underlying plant tissues from DNA damage and oxidative stress?
(A) Carotenes
(B) Xanthophylls
(C) Flavonols
(D) Tannins

2001

**202.** The xanthophyll cycle is a crucial photoprotective mechanism involving the reversible interconversion of:
(A) Lutein and β-carotene
(B) A-tocopherol and gamma-tocopherol
(C) Ascorbate and dehydroascorbate
(D) Violaxanthin, antheraxanthin, and zeaxanthin

2024

**203.** Which of the following acts as the major water-soluble antioxidant in the plant apoplast, acting as a first line of defense against ozone?
(A) Ascorbic acid
(B) A-tocopherol
(C) B-carotene
(D) Zeaxanthin

2018

**204.** Identify the INCORRECT statement regarding non-enzymatic antioxidants.
(A) They can work synergistically with enzymatic antioxidants.
(B) They only operate in the dark.
(C) Tocopherols protect membrane lipids from peroxidation.
(D) Flavonoids can chelate transition metals like iron and copper.

2024

**205.** Provitamin A refers primarily to which plant-synthesized compound?
(A) Ergosterol
(B) B-carotene
(C) Ascorbic acid
(D) A-tocopherol

2024

**206.** Sterol precursors in plants, such as squalene, are biosynthesized by the condensation of units derived from:
(A) Isopentenyl pyrophosphate (IPP) and Dimethylallyl pyrophosphate (DMAPP)
(B) Amino acids
(C) Fatty acids
(D) Simple sugars directly

2011

**207.** Which amino acid serves as the primary precursor for the biosynthesis of numerous alkaloids, including morphine and codeine?
(A) Glycine
(B) Alanine
(C) Tyrosine
(D) Glutamate

2025

**208.** The essential amino acid methionine is synthesized in plants utilizing a carbon backbone derived from:
(A) Pyruvate
(B) A-ketoglutarate
(C) Ribose-5-phosphate
(D) Oxaloacetate / Aspartate

2003

**209.** Lignin, a complex structural polymer in plant cell walls, is derived from precursors generated via the:

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(A) Mevalonate pathway
(B) Pentose phosphate pathway
(C) Shikimate / Phenylpropanoid pathway
(D) TCA cycle

2022

**210.** Match the plant nutrient precursors in List I with their major biosynthetic products in List II.
List I: (Precursor)
P. Tryptophan
Q. Phenylalanine
R. Squalene
S. Geranylgeranyl pyrophosphate (GGPP)
List II: (Product)
1. Phytosterols
2. Auxin (IAA)
3. Carotenoids
4. Flavonoids and Lignin
(A) P-II, Q-IV, R-I, S-III
(B) P-III, Q-IV, R-I, S-II
(C) P-II, Q-I, R-IV, S-III
(D) P-I, Q-II, R-III, S-IV

2012

**211.** Plants synthesize which vital porphyrin ring structure starting from the precursor glutamate (via 5-aminolevulinic acid)?
(A) Sterols
(B) Flavonoids
(C) Carotenoids
(D) Heme and Chlorophyll

2008

**212.** Ergosterol, highly abundant in fungi but also present as a sterol precursor in some plants, acts as a provitamin for:
(A) Vitamin D2
(B) Vitamin A
(C) Vitamin B12
(D) Vitamin K

2023

**213.** Polyamines like putrescine and spermidine, which are involved in stress responses and cell growth, are derived from the amino acids:
(A) Tryptophan and Phenylalanine
(B) Serine and Glycine
(C) Arginine and Ornithine
(D) Leucine and Isoleucine

2004

**214.** Which metabolic pathway produces the erythrose-4-phosphate required for the initial step of the shikimate pathway?
(A) Pentose Phosphate Pathway
(B) Glycolysis
(C) Gluconeogenesis
(D) Glyoxylate Cycle

2010

**215.** The biosynthetic origin of the phytyl side chain of chlorophyll is best traced back to the:
(A) Shikimate pathway
(B) Lipoxygenase pathway
(C) Phenylpropanoid pathway
(D) MEP (non-mevalonate) pathway

2007

**216.** Heat Shock Proteins (HSPs) primarily function as molecular chaperones to:
(A) Directing the synthesis of new genomic DNA.
(B) Assisting in protein folding and preventing aggregation.
(C) Degrading functional and correctly folded host proteins.
(D) Transporting storage carbohydrates across the plasma membrane.

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2004

**217.** Which class of HSPs is strictly ATP-dependent and assists in the folding of nascent polypeptides as they emerge from the ribosome?
(A) sHSPs (small HSPs)
(B) Hsp90
(C) Hsp70
(D) Hsp100

2024

**218.** Small Heat Shock Proteins (sHSPs) differ from the larger Hsp70 and Hsp90 families primarily because they:
(A) Only operate in the mitochondria
(B) Act exclusively as proteases
(C) Do not form oligomeric complexes
(D) Function independently of ATP binding and hydrolysis

2022

**219.** The Hsp100 family (e.g., ClpB in plants) is uniquely known for its ability to:
(A) Disaggregate and resolubilize already aggregated protein complexes
(B) Synthesize de novo proteins
(C) Transport proteins into the nucleus
(D) Covalently modify histone tails

2019

**220.** Which HSP family specifically interacts with steroid hormone receptors and signaling kinases to maintain their conformational stability?
(A) Hsp60

(B) Hsp90
(C) Hsp70
(D) sHSPs

2018

**221.** Under conditions of severe abiotic stress (like heat or drought), the expression of HSP genes is rapidly induced by transcription factors called:
(A) MYB factors
(B) Heat Shock Factors (HSFs)
(C) WRKY factors
(D) NAC domain proteins

2018

**222.** Proteostasis (protein homeostasis) in a plant cell encompasses all of the following EXCEPT:
(A) Random, unregulated protein aggregation
(B) Regulated protein translation
(C) Chaperone-mediated protein folding
(D) Targeted degradation via the ubiquitin-proteasome system

2010

**223.** Hsp60 proteins (chaperonins) typically function by:
(A) Cleaving misfolded proteins into small peptide fragments.
(B) Binding exclusively to specific regulatory DNA promoter sequences.
(C) Providing a secluded environment for protein folding.
(D) Extruding damaged proteins outside the cell membrane boundary.

2007

**224.** Identify the INCORRECT statement regarding plant sHSPs.
(A) They are highly diverse and abundant in plants compared to animals.
(B) They contain a conserved α-crystallin domain.
(C) They assemble into large oligomeric complexes.
(D) They function as ATP-driven unfoldases.

2014

**225.** What provides the energy required for the chaperone function of Hsp70?
(A) GTP hydrolysis
(B) The proton gradient
(C) ATP hydrolysis
(D) NADPH oxidation

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2005

**226.** Molecular chaperones prevent inappropriate interactions among proteins by transiently binding to:
(A) Exposed hydrophilic regions
(B) Exposed hydrophobic residues
(C) Terminal phosphate groups
(D) Carbohydrate moieties

2007

**227.** In the context of proteostasis, if a severely heat-damaged protein cannot be refolded by HSPs, it is typically directed toward:
(A) Secretion into the apoplast
(B) Incorporation into the cell wall
(C) Storage in the central vacuole as a nutrient
(D) Degradation by the 26S proteasome

2008

**228.** Plant anti-viral compounds often work by interfering with the pathogen's ability to:
(A) Replicate its nucleic acids or translate its proteins
(B) Fix carbon dioxide
(C) Synthesize cell wall cellulose
(D) Perform photophosphorylation

2009

**229.** Ribosome-inactivating proteins (RIPs), such as ricin, mediate their toxic effect by:
(A) Phosphorylating ribosomal elongation factors within the cytosol.
(B) Degrading all cellular messenger RNA strands entirely.
(C) Halting translation by cleaving a 28S rRNA adenine residue.
(D) Unfolding nascent polypeptide chains during their synthesis.

2008

**230.** Pathogenesis-related (PR) proteins are defined as plant proteins that are:
(A) Specifically induced in response to pathogen attack or related stress
(B) Constitutively expressed at high levels in all tissues
(C) Primarily responsible for capturing solar energy
(D) Involved solely in normal developmental processes

2021

**231.** Which class of PR proteins exhibits chitinase activity, capable of degrading fungal cell walls?
(A) PR-1
(B) PR-2

(C) PR-5
(D) PR-3

2006

**232.** The PR-5 family of proteins is commonly referred to as:
(A) Defensins
(B) Thaumatin-like proteins
(C) Thionins
(D) Chitinases

2023

**233.** The expression of PR proteins in systemic uninfected leaves following a local infection is a hallmark of:
(A) Hypersensitive response (HR)
(B) Phototropism
(C) Systemic acquired resistance (SAR)
(D) Vernalization

2015

**234.** Pokeweed antiviral protein (PAP) is a well-known example of a:
(A) PR-1 protein
(B) B-1,3-glucanase
(C) Systemin receptor
(D) Type 1 Ribosome-inactivating protein (RIP)

2016

**235.** B-1,3-glucanases (PR-2 proteins) function synergistically with chitinases to defend the plant primarily against:
(A) Fungi
(B) Viroids
(C) Nematodes
(D) Herbivorous insects

2012

**236.** Defensins and thionins are examples of small antimicrobial peptides in plants that typically work by:
(A) Inhibiting viral RNA polymerase
(B) Binding to the pathogen membrane and causing pore formation/leakage
(C) Preventing pathogen DNA methylation
(D) Acting as decoy receptors

2006

**237.** The synthesis of many anti-viral compounds and PR proteins is strongly induced by the accumulation of the signaling molecule:
(A) Salicylic acid
(B) Indole-3-acetic acid
(C) Gibberellic acid
(D) Zeatin

2001

**238.** Identify the INCORRECT statement concerning Ribosome-inactivating proteins (RIPs).
(A) They target and inactivate host DNA polymerases.
(B) They are found in many plant species.
(C) Type II RIPs contain a toxic A-chain and a lectin-like B-chain.
(D) They exhibit highly specific N-glycosidase activity.

2002

**239.** Allosteric regulation of a metabolic enzyme occurs when:
(A) A regulatory molecule binds covalently to the enzyme active site.
(B) An effector binds a non-active site causing conformation change.
(C) The enzyme undergoes rapid degradation by a specific protease.
(D) The substrate concentration exceeds the maximum reaction velocity.

2007

**240.** Feedback inhibition is a regulatory mechanism where:
(A) A downstream metabolic product activates an upstream pathway enzyme.
(B) Multiple different pathways share the same regulatory enzyme unit.
(C) A metabolic product is immediately transported outside the cell.
(D) The first enzyme is inhibited by the final pathway product.

2021

**241.** Reversible covalent modification is a common mechanism of metabolic regulation. The most frequent type of such modification is:
(A) Methylation
(B) Glycosylation
(C) Phosphorylation
(D) Acetylation

2021

**242.** The compartmentalization of metabolic pathways in eukaryotic cells allows for:
(A) Permitting the free mixing of all cellular metabolites and enzymes.
(B) Running opposing pathways simultaneously without futile cycles.
(C) Maintaining a completely uniform pH level across all organelles.
(D) Relying solely on passive diffusion for all enzyme interactions.

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2010

**243.** In allosteric regulation, an effector that decreases the affinity of the enzyme for its substrate is called a:
(A) Positive allosteric modulator
(B) Competitive inhibitor
(C) Suicide inhibitor
(D) Negative allosteric modulator

2002

**244.** Enzymes that undergo phosphorylation for regulation are targeted by specialized enzymes known as:
(A) Kinases
(B) Phosphatases
(C) Mutases
(D) Isomerases

2012

**245.** An example of metabolic compartmentalization is the physical separation of glycolysis and the TCA cycle. Where do these two processes occur?
(A) Both in the cytosol
(B) Glycolysis in mitochondria, TCA cycle in the cytosol
(C) Glycolysis in the cytosol, TCA cycle in the mitochondria
(D) Both in the mitochondria

2022

**246.** Which metabolic phenomenon occurs when an end-product simultaneously inhibits the first enzyme of its own synthesis while activating the first enzyme of an alternative pathway?
(A) Feedback inhibition with cross-activation
(B) Feedforward activation only
(C) Zymogen activation
(D) Isozyme switching

2002

**247.** Zymogen activation is a form of regulation that, unlike phosphorylation, is typically:
(A) Reversible
(B) Allosteric
(C) Controlled strictly by temperature
(D) Irreversible, involving proteolytic cleavage

2008

**248.** The kinetic profile of an allosteric enzyme often deviates from standard Michaelis-Menten kinetics. A graph of reaction velocity versus substrate concentration for such an enzyme typically yields:
(A) A straight line
(B) A sigmoidal curve
(C) A hyperbolic curve
(D) A parabolic curve

2003

**249.** Which of the following is considered the primary advantage of regulatory cascades involving covalent modifications?
(A) They consume minimal ATP.
(B) They only operate in prokaryotes.
(C) They allow for massive signal amplification.
(D) They are completely unaffected by temperature.

2019

**250.** The regulation of sucrose synthesis versus starch synthesis in plant leaves is strongly controlled by the compartmentalization of enzymes between the:
(A) Nucleus and Mitochondria
(B) Endoplasmic Reticulum and Golgi
(C) Vacuole and Cytosol
(D) Cytosol and Chloroplast

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# ANSWERS

**1.** (B) The change in specific rotation accompanying the interconversion of α and β anomers
Monosaccharides in aqueous solution exist as an equilibrium mixture of cyclic α and β anomers. The interconversion between these two forms occurs through an open-chain intermediate. This process causes a shift in the measured optical rotation of the solution over time until equilibrium is reached, a phenomenon known as mutarotation. This behavior is characteristic of all reducing sugars.

**2.** (A) Both A and R are true and R is the correct explanation of A.
Epimers are a specific type of diastereomers that differ in the configuration of only one chiral center. Glucose and mannose are six-carbon sugars that are identical in structure except for the arrangement of the hydroxyl group at the second carbon. Because this single-point difference defines the epimeric relationship, the structural difference accurately explains their specific chemical classification.

**3.** (D) Seliwanoff's test
This biochemical assay differentiates between aldose and ketose sugars based on their rate of dehydration. When heated with concentrated hydrochloric acid and resorcinol, ketoses dehydrate more rapidly to form furfural derivatives. These derivatives then react with resorcinol to produce a characteristic deep cherry-red color, allowing for the specific identification of keto-sugars like fructose within a given sample.

**4.** (A) They are stereoisomers that differ only at the hemiacetal or hemiketal carbon.
When a monosaccharide undergoes cyclization, the carbonyl carbon becomes a new chiral center known as the anomeric carbon. The resulting stereoisomers, designated as α and β, differ solely in the spatial arrangement of the hydroxyl group at this specific carbon atom. This specialized form of isomerism is critical for defining the structural and physical properties of complex carbohydrates.

**5.** (B) D-fructose and D-glucose are C-3 epimers.
Epimers must have the same chemical formula and differ at only one chiral center. Glucose is an aldose, while fructose is a ketose, meaning they are functional isomers rather than epimers. Epimeric relationships exist between glucose and mannose at the second carbon, and between glucose and galactose at the fourth carbon, as they share the same functional groups.

**6.** (B) Aldopentose
Monosaccharides are classified according to the number of carbon atoms and the type of carbonyl group present. Ribose is a five-carbon sugar, which places it in the pentose category. Additionally, it contains an aldehyde functional group at the first carbon. Therefore, ribose is structurally defined as an aldopentose, playing a vital role in nucleic acid formation and metabolism.

**7.** (A) D-form
Stereoisomerism in sugars is determined by the configuration of the asymmetric carbon furthest from the carbonyl group. In biological systems, enzymes are highly stereospecific and primarily interact with the D-isomer of monosaccharides. Consequently, sugars like D-glucose and D-fructose are the most abundant and metabolically active forms found in nature, whereas L-isomers are relatively rare in biological contexts.

**8.** (C) Cuprous oxide
This qualitative test identifies reducing sugars by their ability to reduce copper ions in an alkaline solution. When a reducing sugar is heated with Benedict's reagent, the blue cupric ions are reduced to insoluble cuprous ions. This reaction results in the formation of a brick-red precipitate of cuprous oxide, indicating the presence of a free carbonyl group in the sugar.

**9.** (D) Dihydroxyacetone
An asymmetric carbon atom is a carbon bonded to four different chemical groups, creating chirality. Glyceraldehyde, erythrose, and ribulose all possess at least one such center, making them optically active. Dihydroxyacetone, the simplest

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ketotriose, has a symmetrical structure with two identical hydroxymethyl groups attached to the central carbonyl carbon. Thus, it lacks chirality and is considered an optically inactive molecule.

**10.** (A) P-III, Q-IV, R-I, S-II
Disaccharides are classified by their component sugars and the specific glycosidic bonds linking them. Maltose features an α-1,4 linkage between two glucose units, while lactose contains a β-1,4 bond. Non-reducing sugars like sucrose utilize an α-1,2 linkage, and trehalose uses an α-1,1 bond. Understanding these specific structural connections is essential for identifying the chemical properties and digestibility of carbohydrates.

**11.** (A) Mutarotation
When crystalline α-D-glucopyranose is dissolved in water, the measured specific rotation gradually shifts until it stabilizes at a constant value. This change occurs because the α form spontaneously interconverts with the β form through an open-chain intermediate. The resulting equilibrium mixture of anomers exhibits an optical rotation different from that of the pure starting material, characterizing the mutarotation process.

**12.** (B) β(1→4)
Cellulose is a structural polysaccharide composed of linear chains of D-glucose molecules. Unlike storage starch, which utilizes α-linkages, the glucose units in cellulose are joined by β-1,4-glycosidic bonds. This specific orientation allows the chains to remain straight and form stable, insoluble microfibrils through extensive hydrogen bonding, providing significant mechanical strength to the primary and secondary plant cell walls.

**13.** (B) Glycogen : α(1→6)
Storage polysaccharides like starch and glycogen are composed of glucose units primarily linked by α-1,4 bonds. Branch points in these molecules are created through α-1,6-glycosidic linkages. Glycogen is much more highly branched than amylopectin in starch, allowing for the rapid mobilization of glucose. In contrast, cellulose is a linear structural polymer, and amylose is typically unbranched or minimally branched.

**14.** (A) Pectin
Pectin is a complex structural heteropolysaccharide found in the middle lamella and primary cell walls of plants. Its backbone consists of polygalacturonic acid, which is a polymer of D-galacturonic acid units connected by α-1,4 linkages. This molecule is essential for cell adhesion and provides the gel-like properties necessary for the structural integrity and flexibility of young growing plant tissues.

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**15.** (C) Hemicellulose - Storage in fungi
Polysaccharides serve various biological roles, categorized generally as storage or structural. Starch and glycogen are the primary energy reserves in plants and animals, respectively. Hemicellulose, however, is a structural component found in plant cell walls that cross-links cellulose microfibrils. Fungi typically use chitin for structure and glycogen for energy storage, making the association of hemicellulose with fungal storage incorrect.

**16.** (D) It cross-links cellulose microfibrils to strengthen the cell wall.
Hemicellulose consists of various heteropolysaccharides like xyloglucans and glucomannans. In the plant cell wall, these molecules coat and tether cellulose microfibrils to one another. By forming this interconnected network, hemicellulose contributes to the overall tensile strength and rigidity of the wall. It also helps regulate cell expansion by controlling the spacing and movement of the load-bearing cellulose fibers.

**17.** (A) Extensive intra- and inter-molecular hydrogen bonding
The β-1,4 linkages in cellulose cause every other glucose unit to be flipped, creating a long, straight chain. This arrangement facilitates the formation of numerous hydrogen bonds between the hydroxyl groups of adjacent chains. These strong interactions result in the assembly of tight, crystalline microfibrils that exclude water molecules, making the polymer extremely stable and insoluble in aqueous environments.

**18.** (B) It forms a helical structure that produces a blue-black color with iodine.
Starch is a mixture of two glucose polymers, amylose and amylopectin. Amylose consists of long, unbranched chains that spontaneously coil into a helical shape. When iodine is added, the triiodide ions fit inside these helices, creating a coordination complex with a distinct blue-black color. This reaction serves as a standard

laboratory test to detect the presence of starch in samples.

**19.** (D) Glycogen
Structural polysaccharides provide mechanical support and integrity to organisms, such as cellulose and pectin in plants or chitin in fungi and insects. Hemicellulose also plays a vital structural role by cross-linking cell wall components. Glycogen, however, is a highly branched glucose polymer used exclusively for energy storage in animals and fungi, allowing for the rapid release of glucose.

**20.** (B) I and II
Glycogen is chemically similar to amylopectin but features more frequent branching, occurring every eight to twelve glucose residues. Cellulose molecules are linear and unbranched, forming flat, ribbon-like structures that stack together via hydrogen bonds. While pectin is a plant polysaccharide, it is primarily composed of galacturonic acid rather than xylose and arabinose, which are more characteristic of certain hemicelluloses.

**21.** (C) Pectin
Pectin is a complex carbohydrate found in the cell walls of fruits. When heated with sugar and acid, the pectin molecules form a three-dimensional network that traps water, creating a semi-solid gel. This property is essential for the texture of preserves and jellies. The degree of methylation on the polygalacturonic acid backbone determines the specific gelling conditions and requirements.

**22.** (A) A left-handed single helix
Amylose is the linear component of starch, consisting of glucose units joined by α-1,4-glycosidic bonds. Because of the geometry of these bonds, the chain naturally adopts a coiled, helical conformation rather than a straight line. This single helix is usually left-handed and provides a compact way to store glucose within starch granules, while also allowing for specific interactions with iodine.

**23.** (D) 9,12-octadecadienoic acid
Systematic naming of fatty acids describes the chain length and the position of double bonds. Linoleic acid is an eighteen-carbon chain, indicated by the prefix octadeca. It contains two double bonds, making it dienoic. These unsaturations are located at the ninth and twelfth carbons from the carboxyl end. Therefore, the full systematic name identifies its specific polyunsaturated nature.

**24.** (B) α-Linolenic acid
Humans lack the specific desaturase enzymes required to insert double bonds beyond the ninth carbon of a fatty acid chain. Consequently, polyunsaturated fatty acids like α-linolenic acid, which has double bonds at positions 9, 12, and 15, must be obtained through the diet. This eighteen-carbon omega-3 fatty acid is prevalent in plant sources like flaxseeds, walnuts, and various leafy vegetables.

**25.** (A) Acetyl-CoA → Malonyl-CoA → Acetoacetyl-ACP → β-Hydroxybutyryl-ACP
The biosynthesis of fatty acids begins with the carboxylation of acetyl-CoA to form malonyl-CoA. These units are then transferred to the acyl carrier protein. The first condensation reaction combines acetyl-ACP and malonyl-ACP to produce acetoacetyl-ACP. Subsequent reduction steps convert the ketone group to a hydroxyl group, forming β-hydroxybutyryl-ACP, followed by dehydration and further reduction to elongate the carbon chain.

**26.** (D) Carbon-carbon double bond
Fatty acids are long hydrocarbon chains with a terminal carboxyl group. Saturated fatty acids contain only single bonds between the carbon atoms, allowing the chain to be fully saturated with hydrogen. Unsaturated fatty acids possess one or more carbon-carbon double bonds. This structural feature introduces kinks into the chain, significantly affecting the physical properties, such as lowering the melting point.

**27.** (A) Acetyl-CoA carboxylase
The initial regulated and committed step in the synthesis of long-chain fatty acids is the conversion of acetyl-CoA to malonyl-CoA. This reaction requires bicarbonate and ATP and is catalyzed by the biotin-dependent enzyme acetyl-CoA carboxylase. By producing the three-carbon building block malonyl-CoA, the cell commits metabolic resources to lipid production, making this enzyme a primary target for metabolic control.

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**28.** (B) They serve as major structural components of biological membranes.
Triacylglycerols are neutral lipids formed by esterifying three fatty acid chains to a single glycerol backbone. They are highly efficient energy storage molecules because they are more reduced than carbohydrates. While they are stored in lipid droplets within cells, they do not form the structural bilayer of biological membranes. Membranes are instead composed of amphipathic lipids like phospholipids, glycolipids, and sterols.

**29.** (B) Glycolysis
The synthesis of triacylglycerols requires a glycerol backbone, which is provided in the form of glycerol-3-phosphate. In most tissues, this intermediate is derived from the glycolytic pathway. Specifically, the reduction of dihydroxyacetone phosphate, a triose phosphate intermediate of glycolysis, is catalyzed by glycerol-3-phosphate dehydrogenase. This reaction effectively links carbohydrate metabolism to the storage of lipids as an energy reserve.

**30.** (B) II only
Stearic acid is indeed a fully saturated eighteen-carbon fatty acid. Arachidonic acid is a polyunsaturated fatty acid containing four cis double bonds. Mammals cannot synthesize essential fatty acids de novo due to a lack of certain desaturases. However, while oleic acid is an eighteen-carbon monounsaturated fatty acid, its naturally occurring double bond is in the cis configuration, not the trans configuration.

**31.** (C) Cis double bonds separated by a methylene group
In nature, most unsaturated fatty acids possess double bonds in the cis configuration, which creates a significant bend in the hydrocarbon chain. When multiple double bonds are present, they are typically not conjugated but are instead separated by a methylene group. This non-conjugated arrangement is characteristic of polyunsaturated fatty acids like linoleic and linolenic acid found in plant and animal tissues.

**32.** (B) Increased melting point and decreased solubility in water
As the hydrocarbon chain of a saturated fatty acid becomes longer, the number of Van der Waals interactions between adjacent molecules increases. This requires more thermal energy to disrupt the packing, leading to a higher melting point. Additionally, the increasing size of the non-polar hydrophobic tail overwhelms the polar carboxyl group, significantly reducing the overall solubility of the molecule in water.

**33.** (C) Diacylglycerol acyltransferase (DGAT)
The biosynthesis of triacylglycerols involves the sequential acylation of a glycerol-3-phosphate backbone. After the removal of a phosphate group from phosphatidic acid to form diacylglycerol, a final fatty acid chain must be added. The enzyme diacylglycerol acyltransferase catalyzes this terminal reaction by transferring an acyl group from an acyl-CoA molecule to the third position of diacylglycerol, completing the lipid structure.

**34.** (B) Increasing the proportion of unsaturated fatty acids
Low temperatures can cause biological membranes to lose fluidity and transition into a rigid, gel-like state. To counteract this, cold-adapted plants increase the amount of unsaturated fatty acids in their membrane lipids. The double bonds in these fatty acids create kinks that prevent tight molecular packing. This structural modification lowers the phase transition temperature, ensuring the membrane remains functional and fluid.

**35.** (C) I, II, III, IV
The thylakoid membranes of chloroplasts have a unique lipid composition dominated by non-phospholipids. Monogalactosyldiacylglycerol is the most abundant, making up about half of the total lipid content. It is followed by digalactosyldiacylglycerol. Phosphatidylglycerol is the primary phospholipid present, while sulfoquinovosyldiacylglycerol is typically found in lower amounts, completing the essential lipid matrix required for photosynthetic protein complexes.

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**36.** (A) Sphingolipids
While many lipids like triacylglycerols and phosphoglycerides use glycerol as a structural backbone, sphingolipids are built upon a long-chain amino alcohol called sphingosine. When a fatty acid is attached to the amino group of sphingosine, a ceramide is formed, which serves as the parent compound for all complex sphingolipids. These molecules are essential components of the plasma membranes in eukaryotic cells.

**37.** (C) Stabilizing fluidity by restricting motion while preventing close chain packing
Sterols play a critical role as membrane buffers. At higher temperatures, their rigid four-ring structure restricts the excessive movement of fatty acid chains, preventing the membrane from becoming too fluid. At lower temperatures, sterols interfere with the tight packing and crystallization of the lipid tails. This dual action maintains an optimal range of membrane fluidity, which is vital for proper protein function.

**38.** (A) Phosphatidylcholine
In the plasma membrane of higher plants, phospholipids are the major lipid class. Phosphatidylcholine is consistently the most abundant individual phospholipid, typically accounting for a significant percentage of the total lipid fraction. Other lipids like phosphatidylethanolamine and phosphatidylinositol are present but in smaller quantities. These phospholipids provide the amphipathic matrix necessary to form the stable bilayer structure that defines the cell.

**39.** (D) A carbohydrate moiety attached glycosidically to a lipid backbone
Glycolipids are a class of membrane lipids defined by the presence of one or more sugar residues. These carbohydrate groups, such as glucose or galactose, are linked via a glycosidic bond to either a glycerol or a sphingosine backbone. Unlike phospholipids, they lack a phosphate group. In plants, galactolipids are especially prominent in the membranes of chloroplasts, where they support photosynthesis.

**40.** (A) I, II, III and V
Phospholipids possess both hydrophobic and hydrophilic regions, making them amphipathic. In plants, galactolipids and other glycolipids are mostly concentrated in plastid membranes. Stigmasterol and sitosterol are well-known phytosterols that share a common tetracyclic structure. However, membrane fluidity actually decreases as the ratio of saturated fatty acids increases, because these straight chains pack together more tightly than bent unsaturated chains.

**41.** (C) Amphipathicity leading to the hydrophobic effect
Membrane lipids possess a polar, hydrophilic head and non-polar, hydrophobic tails. When placed in water, the hydrophobic effect drives these molecules to organize such that the tails are shielded from the aqueous environment. This results in the spontaneous formation of a bilayer where the heads face the water and the tails are sequestered in the interior, creating a thermodynamically stable structure.

**42.** (D) Campesterol
Brassinosteroids are a class of polyhydroxylated steroidal plant hormones that regulate growth and development. Their biosynthetic pathway utilizes specific phytosterols as starting materials. Campesterol is the primary precursor that undergoes a series of enzymatic oxidations and reductions to eventually produce brassinolide, the most biologically active brassinosteroid. This pathway is distinct from those used to synthesize other sterols like sitosterol.

**43.** (A) The membrane tends to be in a gel-like solid phase at ambient temperatures.
The phase transition temperature is the point at which a lipid bilayer shifts from a rigid, ordered gel state to a fluid, disordered liquid-crystalline state. A high transition temperature indicates that the lipid tails are tightly packed, often due to high saturation or long chain lengths. Consequently, at normal environmental temperatures, such a membrane remains in a relatively solid, less functional state.

**44.** (B) Cardiolipin : Inner mitochondrial membrane
Specific lipids are often localized to particular organelles to support their function. Cardiolipin is a unique double-phospholipid almost exclusively found in the inner mitochondrial membrane, where it is essential for the stability and activity of the electron transport chain. In contrast, MGDG is a chloroplast lipid, and ergosterol is characteristic of fungal membranes rather than animal or plant cell membranes.

**45.** (B) Fluorescence Recovery After Photobleaching (FRAP)
This imaging technique is widely used to study the mobility of molecules within a bilayer. Lipids or proteins are first tagged with a fluorescent probe. A small area of the membrane is then bleached using a high-intensity laser. The rate at which the fluorescence returns to the bleached spot as unbleached molecules move in allows researchers to calculate the lateral diffusion coefficient.

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**46.** (A) Zwitterion with zero net charge
The isoelectric point is the specific pH at which an amino acid carries no net electrical charge. In this state, the molecule exists as a zwitterion, where the amino group is protonated and positively charged, while the carboxyl group is deprotonated and negatively charged. Because these opposite charges cancel each other out, the amino acid will not migrate in an electric field.

**47.** (C) Canavanine
While plants use twenty standard amino acids for protein synthesis, they also produce hundreds of non-protein amino acids. Canavanine is a structural analog of arginine found in many legumes. Because it mimics arginine, it can be mistakenly incorporated into the proteins of herbivores, leading to dysfunctional proteins. This serves as a potent chemical defense mechanism against predation, unlike the standard proteinaceous residues.

**48.** (D) The α-carboxyl group and the α-amino group
Glycine is the simplest amino acid and lacks an ionizable side chain. During titration, it exhibits two distinct buffering stages. The first region, at low pH, corresponds to the deprotonation of the acidic α-carboxyl group. The second region, occurring at a higher alkaline pH, represents the loss of a proton from the basic α-amino group. These two $pK_a$ values define the molecule's charge.

**49.** (C) Arginine
Amino acids are classified by the chemical nature of their R-groups. Arginine contains a guanidino group in its side chain, which has a very high $pK_a$ value. At physiological pH, this group remains protonated, giving the amino acid a strong positive charge. This makes arginine, along with lysine and histidine, one of the three standard basic amino acids essential for protein structure.

**50.** (A) Both A and R are true and R is the correct explanation of A.
Histidine is uniquely valuable in enzyme catalysis because its imidazole side chain has a $pK_a$ of approximately 6.0. Since this is close to the neutral pH found in many cellular environments, the residue can easily donate or accept protons depending on the local conditions. This versatility allows histidine to participate effectively in acid-base catalysis, justifying its frequent presence in active sites.

**51.** (D) Imino acid
Proline is distinct among the standard protein-building amino acids because its side chain is cyclized back onto the nitrogen of the α-amino group. This structural feature creates a secondary amine, technically making it an imino acid rather than a primary amino acid. This rigid cyclic structure imposes significant constraints on the folding of polypeptide chains, often influencing the formation of secondary structures.

**52.** (A) The nature of the R-group (side chain)
Every amino acid shares a common backbone consisting of an α-carbon, a carboxyl group, and an amino group. The variation in their chemical behavior, such as polarity, charge, and hydrophobicity, is entirely determined by the specific side chain, or R-group, attached to the central carbon. These unique side chains dictate how the amino acid interacts within a protein and with the environment.

**53.** (B) 5.97
The isoelectric point of a simple amino acid without an ionizable side chain is calculated as the arithmetic mean of its two pKa values. For this molecule, adding the $pK_1$ of 2.34 and the $pK_2$ of 9.60 yields a sum of 11.94. Dividing this sum by two results in a $pI$ of 5.97. At this specific pH, the amino acid carries no net charge.

**54.** (A) They are major structural components of plant cell walls.
Non-protein amino acids like GABA and canavanine serve diverse roles in plants, acting as signaling molecules or chemical defenses against herbivores. While they are metabolic intermediates, they are not used by the ribosomes during translation to build proteins. Furthermore, the structural framework of plant cell walls is primarily composed of polysaccharides like cellulose and hemicellulose, rather than these specialized amino acids.

**55.** (D) Methionine and Cysteine
Among the twenty standard amino acids found in proteins, only two contain sulfur atoms. Cysteine possesses a thiol group that can form covalent disulfide bridges, which are vital for stabilizing protein tertiary and quaternary structures. Methionine contains a thioether group and serves

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as the universal starting amino acid for protein synthesis in eukaryotic organisms, playing a key role in methyl group transfers.

**56.** (B) Aromatic amino acids
Proteins absorb ultraviolet light most strongly at a wavelength of 280 nm. This physical property is primarily due to the presence of aromatic amino acids, specifically tryptophan and tyrosine, and to a lesser extent, phenylalanine. The conjugated double bonds in their ring structures undergo electronic transitions when exposed to UV light, providing a convenient method for quantifying protein concentration in laboratory samples.

**57.** (C) Resonance
The peptide bond linking amino acids is formed between the carboxyl carbon of one residue and the amino nitrogen of the next. Resonance between the lone pair on the nitrogen and the carbonyl group creates a hybrid structure. This results in the C-N bond being shorter and stronger than a typical single bond, preventing free rotation and ensuring the peptide unit remains planar.

**58.** (D) Tertiary
Protein structure is organized into four hierarchical levels. The primary level is the sequence of amino acids, while the secondary level involves local folding into helices or sheets. The tertiary structure represents the comprehensive, three-dimensional arrangement of all atoms in a single polypeptide chain. This level of folding is stabilized by various interactions between the side chains of distant amino acid residues.

**59.** (A) The carbonyl oxygen of residue $i$ to the amide nitrogen of residue $i+4$
The α-helix is a common secondary structural motif stabilized by a regular pattern of hydrogen bonding within the polypeptide backbone. Specifically, the oxygen atom of a carbonyl group in one amino acid forms a hydrogen bond with the hydrogen atom attached to the nitrogen of an amino acid located four residues further along the chain. This consistent spacing creates a stable coil.

**60.** (A) P-II, Q-IV, R-III, S-I
The primary structure is defined by the specific sequence of amino acids in a chain. Secondary structure refers to localized folding patterns like α-helices and β-sheets. Tertiary structure describes the complete three-dimensional folding of a single polypeptide, stabilized by side-chain interactions. Finally, quaternary structure involves the spatial arrangement and assembly of multiple polypeptide subunits into a single functional protein complex.

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**61.** (B) $\phi$ (C-α-N) and $\psi$ (C-α-C)
The polypeptide backbone consists of repeating units where rotation is restricted at the peptide bond but allowed around the α-carbon. The two dihedral angles that define the conformation of the backbone are $\phi$, the angle around the N-C-α bond, and $\psi$, the angle around the C-α-C bond. The Ramachandran plot maps the combinations of these angles that are sterically allowed in proteins.

**62.** (A) Proline
Proline is often referred to as a helix breaker because its unique cyclic side chain is covalently bonded to the backbone nitrogen. This structure lacks a hydrogen atom for hydrogen bonding and introduces a rigid bend that disrupts the regular geometry of an α-helix. Additionally, the steric hindrance caused by its ring makes it difficult for the backbone to adopt the necessary helical angles.

**63.** (C) Hydrogen bonds between adjacent strands perpendicular to the chain direction
In an α-helix, hydrogen bonds occur within a single segment of the chain and are parallel to the helix axis. In contrast, a β-pleated sheet is formed by hydrogen bonding between the backbone atoms of different segments or strands of the polypeptide. These bonds are oriented roughly perpendicular to the direction of the chains, linking them together into a stable, sheet-like structure.

**64.** (D) The hydrophobic effect
When a polypeptide folds in an aqueous environment, non-polar side chains tend to cluster together to minimize their contact with water. This phenomenon, known as the hydrophobic effect, is the primary thermodynamic driving force for the folding of globular proteins. It results in the creation of a hydrophobic core sequestered from the solvent, while polar and charged residues remain on the protein surface.

**65.** (C) Free rotation is allowed around the C-N peptide bond.
Due to resonance, the peptide bond between the carbonyl carbon and the amino nitrogen possesses partial double-bond character. This makes the bond rigid and prevents rotation, keeping the six atoms of the peptide group in a single plane. Rotation in the polypeptide backbone is restricted to the bonds connecting the α-carbon to the nitrogen and the carbonyl carbon, known as $\phi$ and $\psi$.

**66.** (B) The spatial arrangement of multiple subunits in a complex.
Many functional proteins are composed of more than one polypeptide chain, referred to as subunits. The quaternary structure describes how these individual subunits are organized and held together by non-covalent interactions or disulfide bonds. This level of organization is essential for the cooperative behavior and regulation of many enzymes and transport proteins, such as hemoglobin or certain plant protein complexes.

**67.** (B) Possessing a hydrogen atom side chain, minimizing steric hindrance.
Glycine is the only achiral amino acid because its side chain consists solely of a single hydrogen atom. This lack of a bulky R-group allows the glycine residue to adopt a much wider range of $\phi$ and $\psi$ dihedral angles without experiencing steric clashes. Consequently, glycine can fit into tight spaces and turns within a protein structure where other amino acids would be restricted.

**68.** (A) Supersecondary structure (motif)
Supersecondary structures, also called motifs, are recurring combinations of secondary structural elements, such as α-helices and β-strands, that form recognizable patterns. A common example is the helix-turn-helix motif, which is frequently found in proteins that bind to DNA. These motifs are more complex than simple helices but are simpler and smaller than independently folding structural units known as protein domains.

**69.** (C) B-DNA
DNA can adopt several different helical forms depending on the environment. B-DNA is the standard, right-handed double helix characterized by distinct major and minor grooves. This conformation is the most stable form under the high-humidity and low-salt conditions typically found within living cells. It serves as the primary structural state for the genetic material in most organisms during normal biological processes.

**70.** (A) I and II
B-DNA is the classical right-handed helix found in physiological conditions. Z-DNA is a unique left-handed form that occurs in sequences with alternating purines and pyrimidines, resulting in a zig-zag backbone. While A-DNA is also a right-handed helix, it is broader and flatter than B-DNA and typically forms under conditions of dehydration or low humidity, rather than high humidity.

**71.** (D) Keto and amino forms
Nitrogenous bases in DNA can exist in different tautomeric forms due to the migration of protons. In the stable environment of the double helix, the bases predominantly adopt the keto form for thymine and guanine, and the amino form for adenine and cytosine. These specific tautomers are essential for maintaining the precise hydrogen-bonding patterns required for accurate base pairing and genetic fidelity.

**72.** (B) Melting temperature (Tm)
When DNA is heated, the hydrogen bonds between the strands break, causing the double helix to separate into single strands. The melting temperature is defined as the midpoint of this transition, where exactly half of the DNA molecules are denatured. This value is a fundamental property used in molecular biology to characterize the stability of DNA sequences and optimize PCR protocols.

**73.** (C) An increase in its melting temperature (Tm)
Guanine-cytosine base pairs are held together by three hydrogen bonds, whereas adenine-thymine pairs have only two. Furthermore, GC pairs have stronger base-stacking interactions compared to AT pairs. Because of this increased stability, DNA molecules with a higher percentage of GC pairs require more thermal energy to denature, resulting in a significantly higher melting temperature than sequences rich in AT pairs.

**74.** (D) Base-stacking interactions involving Van der Waals and hydrophobic forces.
While hydrogen bonds ensure the specificity of base pairing, they are not the primary force stabilizing the double helix. The major thermodynamic contribution comes from the vertical stacking of the planar aromatic bases.

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These interactions, involving Van der Waals forces and the hydrophobic effect, minimize the exposure of the non-polar bases to water, significantly enhancing the overall structural stability of the DNA molecule.

**75.** (A) Uracil : Found in DNA
Nucleic acids are composed of specific nitrogenous bases. Adenine and guanine are purines found in both DNA and RNA. Cytosine is a pyrimidine common to both, while thymine is a methylated pyrimidine typically restricted to DNA. Uracil is the pyrimidine base that replaces thymine in RNA. Therefore, the presence of uracil is a distinguishing feature of RNA molecules rather than DNA.

**76.** (C) The hyperchromic effect
The nitrogenous bases in DNA absorb ultraviolet light at 260 nm. In a double-stranded helix, the close stacking of the bases suppresses this absorption. When the DNA denatures into single strands, the bases are more exposed and free to absorb light, leading to a significant increase in UV absorbance. This phenomenon is a standard method for monitoring the melting of DNA molecules.

**77.** (B) C-1'
A nucleotide is formed by attaching a nitrogenous base to a pentose sugar and a phosphate group. The covalent bond between the sugar and the base is called an N-glycosidic linkage. This bond specifically connects the C-1' carbon of the ribose or deoxyribose sugar to the N-1 position of a pyrimidine or the N-9 position of a purine base.

**78.** (A) The 2'-hydroxyl group on the ribose pentose ring.
The primary chemical difference between RNA and DNA is the type of sugar present. RNA contains ribose, which has a hydroxyl group attached to the second carbon atom. In DNA, this group is replaced by a hydrogen atom, resulting in 2'-deoxyribose. This additional hydroxyl group makes RNA more chemically reactive and susceptible to hydrolysis compared to the more stable DNA.

**79.** (C) A transition mutation during replication
Tautomeric shifts can change the hydrogen-bonding properties of nitrogenous bases. When adenine shifts from its common amino form to the rare imino form, it can incorrectly base-pair with cytosine instead of thymine. If this occurs during DNA replication, it leads to the incorporation of the wrong nucleotide, eventually resulting in a transition mutation where a purine-pyrimidine pair is replaced.

**80.** (B) I, II and IV
Glycolysis is a cytoplasmic pathway that breaks down glucose into pyruvate. Phosphofructokinase-1 is the major rate-limiting enzyme that regulates the flow of metabolites. Pyruvate kinase catalyzes the final irreversible step, producing ATP through substrate-level phosphorylation. While the pathway generates a total of four ATP molecules, two are consumed in the preparatory phase, resulting in a net yield of two ATP.

**81.** (C) Phosphorylation requiring ATP
In the initial step of glycolysis, the enzyme hexokinase transfers a phosphoryl group from a molecule of ATP to the sixth carbon of glucose. This reaction traps the sugar inside the cell by giving it a negative charge and primes it for further metabolic breakdown. Because it consumes energy to activate the substrate, it is a key irreversible and regulated entry point.

**82.** (B) Phosphofructokinase-1 (PFK-1)
Phosphofructokinase-1 is the primary control point of the glycolytic pathway. It is an allosteric enzyme that senses the energy status of the cell. High levels of ATP and citrate act as negative effectors, signalling that the cell has sufficient energy and biosynthetic precursors, thus slowing down glycolysis. Conversely, high levels of AMP and fructose-2,6-bisphosphate activate the enzyme to increase production.

**83.** (D) Dihydroxyacetone phosphate and Glyceraldehyde-3-phosphate
During the cleavage phase of glycolysis, the six-carbon molecule fructose-1,6-bisphosphate is split into two different three-carbon sugars. The enzyme aldolase catalyzes this reversible reaction, producing dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. While both are triose phosphates, only glyceraldehyde-3-phosphate can proceed directly through the payoff phase of glycolysis, requiring the other product to be isomerized by triose phosphate isomerase.

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**84.** (A) Conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate
The oxidative step of glycolysis is catalyzed by glyceraldehyde-3-phosphate dehydrogenase. In this reaction, the aldehyde group of glyceraldehyde-3-phosphate is oxidized while a phosphate group is added. The electrons released during this oxidation are transferred to the coenzyme NAD+, reducing it to NADH. This step is critical because it generates the reducing power that can later be used to produce ATP.

**85.** (C) Ethanol and $CO_2$
When plants experience low oxygen levels, such as during soil flooding, they switch from aerobic respiration to fermentation to maintain ATP production. In most plant tissues, pyruvate is first decarboxylated by pyruvate decarboxylase to form acetaldehyde and $CO_2$. Acetaldehyde is then reduced to ethanol by alcohol dehydrogenase, a process that regenerates the NAD+ needed for glycolysis to continue.

**86.** (A) 2 ATP
The glycolytic pathway consists of two phases: an energy-investment phase and an energy-payoff phase. During the first phase, two ATP molecules are consumed to phosphorylate glucose and fructose-6-phosphate. In the second phase, four ATP molecules are produced via substrate-level phosphorylation. Consequently, the net direct gain of energy from one molecule of glucose under aerobic or anaerobic conditions is two ATP.

**87.** (D) Phosphoglycerate kinase
Most steps in glycolysis are reversible, but three reactions are highly exergonic and serve as irreversible regulatory points: those catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase. In contrast, the reaction catalyzed by phosphoglycerate kinase, which involves the transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate, is readily reversible under physiological conditions within the cell.

**88.** (C) Enolase
Fluoride is a potent inhibitor of the glycolytic enzyme enolase, which catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate. The inhibition occurs because fluoride ions form a complex with magnesium and phosphate at the enzyme's active site, preventing the binding of the substrate. This blocking of glycolysis is often utilized in blood collection tubes to prevent the metabolic breakdown of glucose.

**89.** (D) It completely oxidizes glucose to $CO_2$ and $H_2O$.
The EMP pathway, or glycolysis, is an ancient metabolic process that occurs in the cytosol of almost all living cells and does not require oxygen. It involves an initial investment of two ATP molecules to activate glucose. However, glycolysis only partially oxidizes glucose into two molecules of pyruvate. The complete oxidation to carbon dioxide and water requires subsequent mitochondrial pathways.

**90.** (B) I, III, and V
Fermentation is essential for anaerobic survival because it regenerates the NAD+ consumed during glycolysis. In alcoholic fermentation, pyruvate decarboxylase first removes a carbon as $CO_2$, and then alcohol dehydrogenase reduces the resulting acetaldehyde to ethanol. While this allows for continued ATP production through glycolysis, the total yield is much lower than oxidative phosphorylation. Lactate dehydrogenase is specific to lactic fermentation.

**91.** (A) NADPH and Ribose-5-phosphate
The pentose phosphate pathway is a major metabolic route that diverges from glycolysis at the level of glucose-6-phosphate. Its primary biological function is the production of NADPH, which provides reducing power for biosynthetic reactions like fatty acid synthesis. Additionally, the pathway generates ribose-5-phosphate, which is a necessary building block for the synthesis of nucleotides, RNA, DNA, and various coenzymes.

**92.** (B) Glucose-6-phosphate dehydrogenase
The first step of the oxidative phase in the pentose phosphate pathway is catalyzed by glucose-6-phosphate dehydrogenase. This enzyme oxidizes glucose-6-phosphate to 6-phosphogluconolactone while reducing NADP+ to NADPH. This reaction is irreversible and serves as the primary committed and rate-limiting step of the entire pathway. It is tightly regulated by the cellular ratio of NADPH to NADP+.

**93.** (B) Thiamine pyrophosphate (TPP)
Transketolase is a key enzyme in the non-oxidative phase of the pentose phosphate

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pathway, responsible for transferring two-carbon fragments between sugar phosphates. To perform this function, the enzyme strictly requires thiamine pyrophosphate as a coenzyme. TPP acts as a temporary carrier of the glycoaldehyde unit. This requirement makes transketolase activity a sensitive indicator of thiamine status in biological systems.

**94.** (A) Reductive biosynthetic reactions and antioxidant defense
Unlike NADH, which is primarily used to drive ATP synthesis in mitochondria, the NADPH produced in the pentose phosphate pathway is used for reductive biosynthesis. It provides the high-energy electrons needed for synthesizing fatty acids, cholesterol, and other complex lipids. Additionally, NADPH is vital for maintaining the supply of reduced glutathione, which protects cells against oxidative damage from oxygen radicals.

**95.** (C) It is highly active in tissues undergoing extensive fatty acid synthesis.
The pentose phosphate pathway occurs entirely within the cytosol and shares several intermediates with the glycolytic pathway, such as glucose-6-phosphate and glyceraldehyde-3-phosphate. Because its primary product is NADPH, the pathway is exceptionally active in tissues that require high levels of reducing power for biosynthesis. This includes the liver and adipose tissue in animals, as well as oil production sites.

**96.** (D) Erythrose-4-phosphate
The non-oxidative phase of the pentose phosphate pathway involves a series of sugar-phosphate interconversions catalyzed by transketolase and transaldolase. One unique four-carbon intermediate produced in this process is erythrose-4-phosphate. In plants and bacteria, this molecule is of particular importance as it serves as a critical precursor for the shikimate pathway, which leads to the biosynthesis of aromatic amino acids.

**97.** (C) An epimerase
During the pentose phosphate pathway, ribulose-5-phosphate can be converted into two different pentose isomers. The enzyme ribulose-5-phosphate isomerase converts it into ribose-5-phosphate. Alternatively, the enzyme ribulose-5-phosphate epimerase changes the configuration around the third carbon atom to produce xylulose-5-phosphate. Both products are essential for the subsequent transketolase and transaldolase reactions that link the pathway back to glycolysis for energy.

**98.** (C) 3-carbon fragment
Transaldolase is an enzyme that functions in the non-oxidative phase of the pentose phosphate pathway. It catalyzes the transfer of a three-carbon dihydroxyacetone fragment from a ketose donor, such as sedoheptulose-7-phosphate, to an aldose acceptor, like glyceraldehyde-3-phosphate. This reaction facilitates the conversion of various sugar phosphates and does not require a coenzyme, unlike the two-carbon transfer performed by transketolase.

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**99.** (D) Glucose-6-phosphate and 6-phosphogluconate
The oxidative phase of the pentose phosphate pathway consists of two specific dehydrogenation reactions. First, glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase. Second, after a hydration step, 6-phosphogluconate is oxidized and decarboxylated by 6-phosphogluconate dehydrogenase. Each of these oxidative steps is coupled to the reduction of a molecule of NADP+ to NADPH, providing the cell with essential reducing power.

**100.** (A) Both A and R are true and R is the correct explanation of A.
The pentose phosphate pathway is indispensable for the production of nucleotides required for DNA and RNA synthesis. The oxidative phase converts glucose-6-phosphate into ribulose-5-phosphate, which is then isomerized into ribose-5-phosphate. This five-carbon sugar provides the structural backbone for all purine and pyrimidine nucleotides. Therefore, the pathway's ability to generate this specific intermediate directly supports nucleic acid biosynthesis.

**101.** (C) High levels of NADP+
Glucose-6-phosphate dehydrogenase is the key regulatory enzyme of the pentose phosphate pathway. Its activity is primarily controlled by the availability of its substrate, NADP+. When the cell consumes NADPH for biosynthesis or antioxidant defense, the concentration of NADP+ rises. This increase in NADP+ levels allosterically activates the enzyme, stimulating the pathway to produce more NADPH to restore balance.

**102.** (A) It consumes ATP during the non-oxidative phase.
The pentose phosphate pathway is an efficient metabolic route that generates essential molecules without consuming ATP. The oxidative phase produces NADPH and releases carbon dioxide as a byproduct. The non-oxidative phase consists of a series of reversible sugar interconversions that redistribute carbon atoms to form glycolytic intermediates like fructose-6-phosphate. At no point does the pathway require the input of energy.

**103.** (D) Oxaloacetate
The citric acid cycle begins when a two-carbon acetyl group is transferred from acetyl-CoA to a four-carbon molecule of oxaloacetate. This condensation reaction is catalyzed by the enzyme citrate synthase and results in the formation of the six-carbon molecule citrate. Because oxaloacetate is regenerated at the end of the cycle, it acts as a catalytic acceptor for the incoming acetyl groups.

**104.** (B) Pyruvate dehydrogenase complex
Pyruvate produced in the cytosol by glycolysis is transported into the mitochondrial matrix. There, the pyruvate dehydrogenase complex catalyzes its oxidative decarboxylation into acetyl-CoA. This massive multi-enzyme assembly requires five different coenzymes and serves as the irreversible gatekeeper that regulates the flow of carbon from carbohydrates into the citric acid cycle for energy production or biosynthetic use.

**105.** (C) Function in both catabolic and anabolic pathways.
The citric acid cycle is termed amphibolic because it serves dual purposes in metabolism. It acts catabolically by oxidizing acetyl-CoA to release energy and $CO_2$. Simultaneously, it functions anabolically by providing carbon skeletons for the synthesis of other molecules. For example, α-ketoglutarate and oxaloacetate are precursors for amino acids, while succinyl-CoA is essential for the biosynthesis of porphyrins.

**106.** (D) Succinate dehydrogenase
Most enzymes of the citric acid cycle are soluble proteins located within the mitochondrial matrix. Succinate dehydrogenase is the unique exception, as it is an integral protein embedded in the inner mitochondrial membrane. This dual-function enzyme also serves as Complex II of the electron transport chain, directly transferring electrons from the oxidation of succinate to the ubiquinone pool during respiration.

**107.** (C) 3
A single turn of the citric acid cycle involves three specific oxidative steps where NAD+ is reduced to NADH. These reactions are catalyzed by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase. Consequently, for every acetyl-CoA molecule that enters the cycle, three molecules of NADH are generated, representing a significant portion of the energy harvested from the oxidation of the acetyl group.

**108.** (B) Succinyl-CoA synthetase (thiokinase)
The conversion of succinyl-CoA to succinate is the only step in the citric acid cycle that directly produces a high-energy phosphate bond. This reaction, catalyzed by succinyl-CoA synthetase, uses the energy released from breaking the thioester bond to drive the phosphorylation of GDP to GTP or ADP to ATP. This process is a clear example of substrate-level phosphorylation occurring within mitochondria.

**109.** (B) Succinyl-CoA
Succinyl-CoA is a high-energy intermediate of the citric acid cycle that serves as a critical starting point for various biosynthetic pathways. In plants and animals, it condenses with glycine to begin the synthesis of porphyrins. These nitrogen-containing ring structures are the fundamental building blocks for essential molecules such as heme in cytochromes and chlorophyll in plants, which is necessary for photosynthesis.

**110.** (A) P-III, Q-I, R-IV, S-II
Citrate synthase produces citrate from acetyl-CoA and oxaloacetate. Aconitase then rearranges citrate into isocitrate. Isocitrate dehydrogenase performs an oxidative decarboxylation to yield α-ketoglutarate. Later in the cycle, the enzyme fumarase hydrates fumarate to form malate. Recognizing these specific enzyme-product pairings is essential for understanding the sequence of chemical transformations that occur during the complete oxidation of carbon in mitochondria.

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**111.** (C) Malonate
Malonate is a three-carbon dicarboxylic acid that structurally resembles the four-carbon substrate succinate. Due to this similarity, it acts as a classical competitive inhibitor of the enzyme succinate dehydrogenase. By binding to the active site, malonate blocks the oxidation of succinate to fumarate, effectively halting the citric acid cycle. This inhibition was historically important in elucidating the cyclic nature of this pathway.

**112.** (D) Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase
The citric acid cycle removes the two carbons of the entering acetyl group by releasing them as carbon dioxide. This process occurs during two successive oxidative decarboxylation reactions. First, isocitrate is converted to α-ketoglutarate, and then α-ketoglutarate is converted to succinyl-CoA. Each of these steps is catalyzed by a specific dehydrogenase and results in the production of one molecule of $CO_2$.

**113.** (C) Aconitase
Fluoroacetate is a potent metabolic poison that undergoes lethal synthesis within the cell. It is first converted into fluoroacetyl-CoA and then condensed with oxaloacetate to form fluorocitrate. This molecule acts as a suicide inhibitor of the enzyme aconitase. By blocking the conversion of citrate to isocitrate, fluorocitrate halts the citric acid cycle, leading to a toxic accumulation of citrate and energy failure.

**114.** (B) Phosphorylation/Dephosphorylation
The activity of the pyruvate dehydrogenase complex is tightly controlled to balance carbohydrate oxidation and biosynthesis. In plants, the primary regulatory mechanism is reversible phosphorylation. A specific kinase phosphorylates the enzyme complex, rendering it inactive, while a phosphatase removes the phosphate group to restore activity. This system allows the plant to rapidly adjust mitochondrial respiration in response to light and metabolic demand.

**115.** (D) The synthesis of glucose from non-carbohydrate precursors.
Gluconeogenesis is a vital metabolic pathway that allows organisms to produce glucose when carbohydrate supplies are low. It utilizes non-carbohydrate precursors, such as pyruvate, lactate, glycerol, and certain amino acids. While it shares many enzymes with glycolysis, it is not a simple reversal of that pathway. Instead, it employs unique enzymes to bypass the three irreversible steps of glycolysis.

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**116.** (A) Glyoxysome
In germinating oilseeds, stored triacylglycerols are the primary energy source. The process of converting these lipids into carbohydrates begins in specialized peroxisomes called glyoxysomes. Within these organelles, fatty acids are broken down through β-oxidation to produce acetyl-CoA. This acetyl-CoA then enters the glyoxylate cycle, allowing the seeds to produce four-carbon intermediates that can eventually be used for gluconeogenesis in the cytosol.

**117.** (C) Isocitrate lyase and Malate synthase
The glyoxylate cycle is a modification of the citric acid cycle that bypasses the two decarboxylation steps where $CO_2$ is normally lost. This is achieved through two unique enzymes. Isocitrate lyase splits isocitrate into succinate and glyoxylate. Malate synthase then condenses glyoxylate with a second molecule of acetyl-CoA to form malate. These enzymes allow for the net synthesis of carbohydrates.

**118.** (A) Succinate
In one turn of the glyoxylate cycle, two molecules of acetyl-CoA enter the pathway. One is condensed with oxaloacetate to form citrate, which is then converted to isocitrate. Isocitrate is cleaved to yield glyoxylate and succinate. The glyoxylate then reacts with the second acetyl-CoA to regenerate malate and subsequently oxaloacetate. Thus, the net product exported from the cycle is succinate.

**119.** (D) Pyruvate carboxylase and PEP carboxykinase.
In glycolysis, the conversion of phosphoenolpyruvate to pyruvate is irreversible. To reverse this during gluconeogenesis, the cell uses a two-step bypass. First, pyruvate carboxylase converts pyruvate into oxaloacetate within mitochondria. Second, phosphoenolpyruvate carboxykinase converts oxaloacetate into phosphoenolpyruvate. This detour requires energy in the form of ATP and GTP, allowing the pathway to overcome the original thermodynamic barrier.

**120.** (B) I and II
The glyoxylate cycle is a specialized pathway found in the glyoxysomes of fatty seeds, enabling the conversion of lipids to sugars. Succinate produced in this cycle is moved to the mitochondria to enter the citric acid cycle for further processing. Gluconeogenesis, however, is a highly energy-consuming process that requires several molecules of ATP and GTP to drive the synthesis of glucose.

**121.** (C) Oxaloacetate
During the mobilization of lipids in germinating seeds, succinate from the glyoxysome is converted to malate in the mitochondria. This malate is then transported into the cytosol. Once in the cytoplasm, the enzyme malate dehydrogenase oxidizes the malate back into oxaloacetate. This cytosolic oxaloacetate then serves as the substrate for phosphoenolpyruvate carboxykinase, continuing the upward flow of carbon through gluconeogenesis.

**122.** (D) Fructose-1,6-bisphosphatase
One of the major regulatory hurdles in gluconeogenesis is bypassing the irreversible phosphofructokinase-1 reaction of glycolysis. This is achieved by the enzyme fructose-1,6-bisphosphatase, which catalyzes the hydrolytic removal of a phosphate group from the first carbon of fructose-1,6-bisphosphate. This reaction produces fructose-6-phosphate and inorganic phosphate, allowing the metabolic flow to proceed toward the synthesis of glucose-6-phosphate and free glucose.

**123.** (A) Acetate or fatty acids as the sole carbon source.
The glyoxylate cycle allows organisms to utilize two-carbon compounds, such as acetate or acetyl-CoA derived from fatty acid breakdown, for the net synthesis of carbohydrates. By bypassing the $CO_2$-releasing steps of the TCA cycle, these organisms can preserve carbon atoms to build essential four-carbon intermediates. This metabolic flexibility is crucial for germinating oilseeds and certain bacteria growing on simple sources.

**124.** (B) Converting stored fats to carbohydrates for fueling seedling growth.
The primary biological role of the glyoxylate cycle in plants is to bridge the gap between lipid catabolism and carbohydrate biosynthesis. During germination, oilseeds rely on stored triacylglycerols for energy. The glyoxylate cycle converts the resulting acetyl-CoA into succinate, which is then used in gluconeogenesis to produce the sugars necessary for the developing seedling until it becomes photosynthetically self-sufficient.

**125.** (B) GTP or ATP depending on the organism/ enzyme variant.
The conversion of oxaloacetate to phosphoenolpyruvate is a critical energy-requiring step in gluconeogenesis. The enzyme PEP carboxykinase catalyzes this reaction by simultaneously decarboxylating oxaloacetate and phosphorylating it. Depending on the specific organism and the cellular location of the enzyme, the high-energy phosphate group is donated by either GTP or ATP. This investment of energy drives the reaction.

**126.** (A) Molecular oxygen
The mitochondrial electron transport chain consists of a series of redox centers that transfer electrons from high-energy carriers like NADH and FADH2 to a final acceptor. This process occurs through four major protein complexes and mobile carriers. The ultimate destination for these electrons is molecular oxygen, which is reduced to form water. This final step is essential for respiration.

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**127.** (C) Complex I (NADH dehydrogenase)
Complex I, also known as NADH:ubiquinone oxidoreductase, is the largest complex in the mitochondrial electron transport chain. It catalyzes the transfer of two electrons from NADH to the mobile lipid carrier ubiquinone. This reaction is coupled to the translocation of four protons from the mitochondrial matrix into the intermembrane space, contributing significantly to the establishment of the electrochemical proton gradient.

**128.** (D) Mobile lipid-soluble electron carrier.
Coenzyme Q, or ubiquinone, is a small hydrophobic molecule found within the phospholipid bilayer of the inner mitochondrial membrane. Because of its lipid solubility, it can move freely between the large, stationary protein complexes. Its primary role is to accept electrons from both Complex I and Complex II and deliver them to Complex III, acting as a vital bridge.

**129.** (C) Ubiquinone pool
In plant mitochondria, the alternative respiratory pathway provides an exit for electrons that bypasses the main cytochrome pathway. The alternative oxidase enzyme branches directly from the ubiquinone pool. Instead of passing electrons through Complexes III and IV, AOX transfers them directly from ubiquinol to molecular oxygen. This shortcut is not coupled to proton pumping, meaning energy is dissipated as heat.

**130.** (B) I, III and IV
Complex IV is the terminal oxidase of the standard respiratory chain that reduces oxygen to water. Complex II transfers electrons from succinate but does not possess the mechanism to pump protons across the membrane. The alternative oxidase pathway dissipates the energy of electron transfer as heat rather than storing it as ATP. Unlike Complex IV, AOX is notably resistant to cyanide.

**131.** (D) Cyanide
Cyanide is an extremely toxic chemical that acts by binding with very high affinity to the iron atom in the heme group of cytochrome c oxidase, also known as Complex IV. This binding prevents the transfer of electrons to molecular oxygen, effectively halting the entire electron transport chain. As a result, ATP production via oxidative phosphorylation ceases, leading to rapid energy failure.

**132.** (A) Generating heat to volatilize scents to attract pollinators.
In certain plants, such as Arum lilies, the alternative oxidase pathway is highly active during flowering. Because AOX bypasses the proton-pumping sites of the main electron transport chain, the energy of electron transfer is released entirely as thermal energy. This rapid heat production raises the temperature of the floral tissue, which helps evaporate volatile odoriferous compounds that attract insect pollinators.

**133.** (C) Cytochrome c
Cytochrome c is a small hemeprotein that serves as a mobile electron carrier in the respiratory chain. Unlike ubiquinone, which is lipid-soluble and moves within the membrane, cytochrome c is water-soluble and resides in the intermembrane space. It functions by accepting a single electron from Complex III and transporting it to Complex IV, where it is used to reduce oxygen.

**134.** (A) Complex I
Rotenone is a naturally occurring compound that acts as a potent and specific inhibitor of Complex I in the mitochondrial electron transport chain. It binds to the ubiquinone binding site of the enzyme, blocking the transfer of electrons from the iron-sulfur clusters to ubiquinone. This prevents the oxidation of NADH and disrupts the establishment of the proton gradient, limiting ATP synthesis.

**135.** (D) Protons
As electrons travel through Complexes I, III, and IV of the electron transport chain, the energy released from these redox reactions is used to pump hydrogen ions, or protons, from the mitochondrial matrix into the intermembrane space. This creates a significant concentration and electrical gradient across the inner membrane. The resulting proton-motive force is harnessed by ATP synthase.

**136.** (D) It is highly sensitive and completely inhibited by potassium cyanide.
The alternative oxidase pathway is a unique feature of plant respiration that allows electrons to bypass the cyanide-sensitive Complex IV. Consequently, AOX-mediated respiration is characteristically resistant to cyanide inhibition. While it reduces the efficiency of ATP production by bypassing several proton-pumping sites, it plays a vital role in managing metabolic stress and preventing the formation of harmful oxygen species.

**137.** (C) Chemiosmotic theory
The link between the electron transport chain and ATP synthesis is explained by the chemiosmotic theory. This model proposes that the energy from electron flow is used to create a proton gradient across the inner mitochondrial membrane. The return of these protons to the matrix through the ATP synthase complex provides the mechanical energy necessary to catalyze the phosphorylation of ADP to ATP.

**138.** (D) Electrochemical proton gradient known as the proton-motive force.
During respiration, the electron transport chain pumps protons into the intermembrane space, creating a higher concentration of hydrogen ions

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and a more positive charge compared to the matrix. This electrochemical potential, termed the proton-motive force, acts like a battery. It stores the energy harvested from food molecules until it can be used by the ATP synthase complex to drive production.

**139.** (A) Rotational catalysis
The $F_1F_0$-ATP synthase is a complex molecular machine that converts the energy of a proton gradient into chemical energy. As protons flow through the membrane-embedded $F_0$ sector, they cause a central stalk to rotate. This physical rotation induces a sequence of conformational changes in the catalytic β subunits of the $F_1$ sector, enabling them to bind substrates, synthesize ATP, and release it.

**140.** (A) I, II, III and IV
The $F_1F_0$-ATP synthase consists of two functional domains: $F_0$ for proton translocation and $F_1$ for catalysis. The rotation of the gamma subunit within the $F_1$ sector is driven by the proton flow, altering the binding sites to facilitate ATP production. Oligomycin blocks this process by binding to the $F_0$ unit. While its primary role is synthesis, the enzyme can also hydrolyze ATP.

**141.** (B) Peter Mitchell
The concept that a transmembrane electrochemical gradient of protons drives the synthesis of ATP was first proposed by Peter Mitchell in 1961. This revolutionary idea, known as the chemiosmotic hypothesis, unified the fields of metabolism and bioenergetics. Despite initial skepticism from the scientific community, his work was eventually confirmed and earned him the Nobel Prize in Chemistry in 1978.

**142.** (A) 2,4-Dinitrophenol (DNP).
An uncoupler is a chemical that dissociates electron transport from ATP synthesis. 2,4-Dinitrophenol is a lipophilic weak acid that can easily cross the inner mitochondrial membrane while carrying a proton. By shuttling protons back into the matrix, DNP bypasses ATP synthase and dissipates the proton-motive force. Consequently, oxygen consumption continues at a high rate, but no ATP is produced, releasing heat.

**143.** (C) Open, Loose, Tight (O, L, T).
According to the binding-change mechanism of ATP synthase, the three catalytic β subunits cycle through three distinct conformations. In the Open state, ATP is released and new substrates can enter. In the Loose state, ADP and inorganic phosphate are loosely trapped. In the Tight state, the substrates are squeezed together to form ATP. The rotation of the gamma subunit drives these transitions.

**144.** (D) Transferring a phosphoryl group from a high-energy substrate to ADP.
Substrate-level phosphorylation is a direct method of ATP production where a phosphate group is transferred from a high-energy metabolic intermediate directly to ADP. This process does not require oxygen or a proton gradient and occurs during glycolysis and the citric acid cycle. In contrast, oxidative phosphorylation is indirect, relying on the electron transport chain and chemiosmosis to power ATP synthase.

**145.** (C) Continued oxygen consumption but halted ATP synthesis
Adding an uncoupling agent like DNP collapses the electrochemical proton gradient without inhibiting the electron transport chain itself. Because the respiratory chain is no longer restricted by the buildup of protons in the intermembrane space, electron flow and oxygen consumption actually increase. However, since the proton-motive force is gone, ATP synthase can no longer function, and the synthesis of ATP stops.

**146.** (B) The c-ring of the $F_0$ sector.
The $F_0$ sector of the ATP synthase complex is located within the inner mitochondrial membrane. It contains a ring of c-subunits that act as the rotor of the molecular motor. Protons entering through the a-subunit are transferred to the c-ring, causing it to spin as they move toward the matrix. This rotation is directly transmitted to the central stalk, which drives catalysis.

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**147.** (B) 2.5
The number of ATP molecules produced per NADH oxidized is determined by the number of protons pumped by the electron transport chain and the number of protons required by ATP synthase. For every pair of electrons from NADH, ten protons are pumped across the membrane. Given the stoichiometry of modern ATP synthase models, this results in the production of approximately 2.5 ATP molecules.

**148.** (A) Protons are pumped from the intermembrane space into the matrix by the ETC.
Oxidative phosphorylation relies on the establishment of a proton gradient across the inner mitochondrial membrane. During electron transport, the complexes pump protons out of the matrix and into the intermembrane space, creating a high concentration of hydrogen ions. The subsequent flow of these protons back into the matrix, through the ATP synthase complex, provides the necessary energy to synthesize ATP.

**149.** (C) Antioxidant cofactor involved in collagen synthesis.
Vitamin C, or ascorbic acid, is an essential water-soluble nutrient that acts as a powerful reducing agent. It serves as a necessary cofactor for the enzymes prolyl and lysyl hydroxylase, which are required for the proper cross-linking and stabilization of collagen fibers. Furthermore, it plays a vital role in the cellular antioxidant defense system by scavenging reactive oxygen species and regenerating antioxidants.

**150.** (A) Both A and R are true and R is the correct explanation of A.
Vitamin A is critical for the maintenance of healthy vision. It is converted into retinal, a light-sensitive molecule that combines with the protein opsin to form rhodopsin in the rod cells of the retina. Rhodopsin is essential for detecting light in low-intensity conditions. A deficiency in Vitamin A prevents the adequate formation of this pigment, leading to impaired night vision.

**151.** (D) Vitamin E (Tocopherol)
Vitamin E is a group of fat-soluble compounds, with $\alpha$-tocopherol being the most biologically active. Because of its lipophilic nature, it resides within the hydrophobic interior of cellular membranes. Its primary function is to protect these membranes from oxidative damage by neutralizing lipid peroxyl radicals. This prevents the chain reaction of lipid peroxidation, which would otherwise compromise the structural integrity.

**152.** (B) Oxidative decarboxylation
Thiamine, in its active coenzyme form as thiamine pyrophosphate, is essential for several key metabolic reactions. It facilitates the cleavage of carbon-carbon bonds adjacent to a carbonyl group. This is particularly important in the oxidative decarboxylation of $\alpha$-keto acids, such as the conversion of pyruvate to acetyl-CoA. Without thiamine, these critical steps in energy metabolism are severely impaired.

**153.** (C) Gamma-carboxylation of clotting factors.
Vitamin K is an essential fat-soluble cofactor for the enzyme gamma-glutamyl carboxylase. This enzyme adds a carboxyl group to specific glutamic acid residues on several blood clotting proteins, including prothrombin and Factors VII, IX, and X. This modification creates calcium-binding sites that are necessary for the proteins to adhere to phospholipid surfaces during the coagulation cascade, allowing for effective clot formation.

**154.** (D) Niacin (B3)
Pellagra is a nutritional deficiency disease characterized by the three Ds: dermatitis, diarrhea, and dementia. It is caused by a severe lack of niacin or its precursor amino acid, tryptophan. Niacin is essential for the synthesis of the coenzymes NAD and NADP, which are involved in hundreds of redox reactions across all major metabolic pathways. Consequently, its deficiency affects various tissues.

**155.** (A) Niacin
Niacin, also known as Vitamin B3, is the dietary precursor for the nicotinamide moiety of the vital coenzymes nicotinamide adenine dinucleotide and its phosphorylated form. These molecules are the primary electron carriers in the cell, facilitating the transfer of hydride ions during catabolic and anabolic reactions. Because they are central to energy metabolism and biosynthesis, maintaining adequate niacin is essential.

**156.** (D) Amino acid metabolism (e.g., transamination)
The active form of Vitamin B6, pyridoxal phosphate, is an exceptionally versatile coenzyme primarily involved in nitrogen metabolism. It serves as a necessary cofactor for various enzymes, most notably transaminases, which transfer amino groups between amino acids and keto acids. Additionally, PLP is required for decarboxylation and deamination reactions, making it a central player in the synthesis and breakdown of proteinaceous compounds.

**157.** (B) Vitamin D
Vitamin D is unique among vitamins because it can be synthesized endogenously by the

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body. When skin is exposed to ultraviolet B radiation from sunlight, a sterol precursor called 7-dehydrocholesterol is converted into cholecalciferol. This molecule then undergoes further hydroxylation in the liver and kidneys to become the active hormone calcitriol, which is essential for regulating calcium and phosphorus levels.

**158.** (A) Coenzyme A
Pantothenic acid is a water-soluble vitamin that serves as a vital structural unit for the synthesis of coenzyme A. CoA is a ubiquitous molecule required for the metabolism of carbohydrates, lipids, and amino acids. It functions as a carrier of acyl groups, which are attached via a high-energy thioester bond. This makes CoA central to the citric acid cycle and fatty acid oxidation.

**159.** (C) Vitamin B12 : Night blindness
Vitamin C deficiency leads to scurvy, characterized by weakened connective tissue, while Vitamin D deficiency causes rickets in children. Vitamin B1 deficiency results in beriberi, affecting the nervous and cardiovascular systems. However, night blindness is specifically caused by a deficiency of Vitamin A, which is essential for visual pigment formation. Vitamin B12 deficiency instead typically leads to megaloblastic anemia.

**160.** (A) P-II, Q-III, R-IV, S-I
Plant hormones are synthesized from specific metabolic precursors. Auxin is primarily derived from the amino acid tryptophan. Gibberellins are produced through the diterpene pathway starting from geranylgeranyl pyrophosphate. Cytokinins are synthesized from adenine or isopentenyl pyrophosphate. Ethylene is unique as it is a gaseous hormone produced from the amino acid methionine via the intermediate 1-aminocyclopropane-1-carboxylic acid.

**161.** (C) Mevalonate and MEP (terpenoid) pathways
Gibberellins are a large family of tetracyclic diterpene acids. Their biosynthesis follows the general terpenoid pathway, utilizing five-carbon building blocks. In plants, these units are produced in both the cytosol via the mevalonate pathway and in plastids via the methylerythritol phosphate pathway. The synthesis involves the formation of ent-kaurene within the chloroplast, followed by sequential oxidations on the endoplasmic reticulum.

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**162.** (B) PIN proteins
Auxin is unique among plant hormones for its directed, polar transport through tissues. This movement is facilitated by specific transport proteins. PIN-FORMED proteins are auxin efflux carriers that are typically localized to one side of the cell membrane. By controlling the placement of these proteins, the plant can steer the flow of auxin, which is essential for processes like tropisms.

**163.** (D) Aux/IAA proteins
In the absence of auxin, gene expression is blocked by a family of repressor proteins known as Aux/IAA proteins. These repressors bind to and inhibit auxin response factors. When auxin levels rise, the hormone acts as a molecular glue, bringing the Aux/IAA proteins to a specific ubiquitin ligase complex. This leads to the degradation of the repressors, allowing gene transcription.

**164.** (A) TIR1
The primary receptor for auxin in plant cells is the Transport Inhibitor Response 1 protein. TIR1 is a component of an SCF-type E3 ubiquitin ligase complex. When auxin binds to the pocket of TIR1, it enhances the affinity of the receptor for its target repressor proteins, the Aux/IAA family. This binding triggers the ubiquitination and subsequent destruction of the repressors.

**165.** (C) Promoting cell division.
Cytokinins are a class of plant hormones that were originally identified by their ability to stimulate cytokinesis. They play a critical role in promoting cell division in both the roots and shoots. Additionally, cytokinins are involved in delaying leaf senescence, regulating nutrient mobilization, and influencing the development of lateral buds. Their interaction with auxin determines overall plant architecture and growth.

**166.** (A) A-amylase
During seed germination, the embryo produces gibberellins which diffuse to the aleurone layer. There, the hormone triggers the synthesis and secretion of various hydrolytic enzymes. The most prominent among these is α-amylase, which breaks down the starch stored in the endosperm into simple sugars. These sugars provide the energy and carbon skeletons required for the rapid growth of the developing seedling.

**167.** (D) GID1
The perception of gibberellins is mediated by a soluble protein called GIBBERELLIN INSENSITIVE DWARF$_1$. When GA binds to the GID1 receptor, the complex undergoes a conformational change that allows it to interact with DELLA proteins, which are growth-repressing factors. This interaction leads to the ubiquitination and degradation of the DELLA proteins, thereby relieving the inhibition of growth-promoting genes.

**168.** (B) Shikimate pathway
The shikimate pathway is the primary route for the synthesis of aromatic amino acids in plants, bacteria, and fungi. It converts simple carbohydrate precursors from glycolysis and the pentose phosphate pathway into phenylalanine, tyrosine, and tryptophan. Because tryptophan is the major precursor for the de novo biosynthesis of indole-3-acetic acid, the shikimate pathway is fundamental for maintaining the supply of auxin.

**169.** (C) Two-component phosphorelay system
Cytokinin perception and signaling utilize a complex system modeled after bacterial two-component systems. It involves membrane-bound histidine kinase receptors that undergo autophosphorylation upon hormone binding. The phosphate group is then transferred through histidine phosphotransfer proteins to response regulators in the nucleus. These response regulators act as transcription factors, either activating or repressing the expression of cytokinin-responsive genes.

**170.** (D) Zeatin
While several compounds exhibit cytokinin activity, zeatin is considered the most potent and widespread naturally occurring cytokinin in higher plants. It was first isolated from immature maize kernels. Zeatin exists in both cis and trans isomeric forms, with trans-zeatin usually being the more biologically active variant. Along with other isoprenoid cytokinins, it is essential for regulating cell division throughout development.

**171.** (A) A C40 carotenoid precursor (e.g., zeaxanthin).
Abscisic acid is synthesized in plants through the indirect carotenoid pathway. The process starts with the synthesis of C40 carotenoids like zeaxanthin in the plastids. These molecules are then converted into violaxanthin and subsequently neoxanthin. A critical rate-limiting step involves the oxidative cleavage of these C40 precursors by the enzyme NCED to produce a C15 intermediate called xanthoxin.

**172.** (B) ACC oxidase (ACO)
Ethylene biosynthesis is a two-step pathway starting from the precursor S-adenosylmethionine. First, ACC synthase converts SAM into 1-aminocyclopropane-1-carboxylic acid. In the second and final step, the enzyme ACC oxidase catalyzes the oxidation of ACC to produce ethylene, carbon dioxide, and hydrogen cyanide. This reaction requires oxygen and ascorbate as co-factors and is a key point of regulation.

**173.** (B) Linolenic acid via the lipoxygenase (LOX) pathway.
Jasmonates are oxygenated lipid derivatives that function as vital signaling molecules in plant defense and development. Their biosynthesis begins with the release of α-linolenic acid from membrane lipids. The lipoxygenase pathway then converts this fatty acid through a series of enzymatic steps. Subsequent β-oxidation cycles in the peroxisome shorten the carbon chain to produce the active hormone, jasmonic acid.

**174.** (A) Isochorismate pathway and/or Phenylalanine ammonia-lyase (PAL) pathway.
Salicylic acid is a phenolic hormone essential for plant immunity. It can be synthesized through two distinct pathways originating from the shikimate pathway product chorismate. The isochorismate pathway involves the conversion of chorismate to isochorismate. Alternatively, the PAL pathway converts phenylalanine into cinnamic acid, which is then processed into SA. Both pathways contribute to the total pool of salicylic acid.

**175.** (B) Methionine → SAM → ACC → Ethylene.
The biosynthesis of ethylene follows a well-defined sequence known as the Yang cycle. It begins with the amino acid methionine, which is converted into S-adenosylmethionine by SAM synthetase. Next, the enzyme ACC synthase produces 1-aminocyclopropane-1-carboxylic acid from SAM. Finally, ACC oxidase converts this intermediate into gaseous ethylene. This pathway allows the plant to precisely control ethylene levels.

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**176.** (D) Cell expansion, elongation, and resistance to environmental stress.
Brassinosteroids are steroidal hormones that are essential for nearly all aspects of plant growth and development. They are particularly known for promoting cell expansion and elongation, often working in conjunction with auxin. Beyond growth, brassinosteroids enhance the plant's ability to tolerate various abiotic stresses, such as cold and drought. They also play roles in vascular differentiation and regulation.

**177.** (C) Inducing stomatal closure to minimize transpirational water loss.
Abscisic acid is often called the stress hormone due to its central role in the plant's response to environmental challenges. During drought, ABA levels rise rapidly in the leaves. The hormone triggers a signaling cascade in the guard cells that leads to the efflux of potassium and chloride ions. This loss of turgor causes the stomata to close, reducing transpiration.

**178.** (B) Salicylic Acid
Systemic Acquired Resistance is a broad-spectrum, long-lasting defense response that occurs in the uninfected parts of a plant following an initial pathogen attack. Salicylic acid is the indispensable signaling molecule for the establishment and maintenance of SAR. It triggers the expression of many pathogenesis-related genes throughout the plant, effectively priming the entire organism against future infections by various pathogens.

**179.** (D) Endoplasmic reticulum.
Unlike many hormone receptors that are located on the plasma membrane, the receptors for ethylene, such as ETR1, are primarily localized to the membrane of the endoplasmic reticulum. These receptors act as negative regulators of the ethylene response. In the absence of ethylene, they activate a kinase that prevents signaling. When ethylene binds to the receptor, it inactivates this inhibitory pathway.

**180.** (A) Jasmonates
JASMONATE ZIM-domain proteins are the key negative regulators of the jasmonate signaling pathway. In the absence of the hormone, JAZ proteins bind to and repress transcription factors like MYC2. When the bioactive jasmonate signal is present, it promotes the interaction between JAZ proteins and a specific ubiquitin ligase complex. This leads to the degradation of the JAZ repressors, releasing transcription factors.

**181.** (C) BZR1 and BES1
The brassinosteroid signaling pathway involves a cascade of phosphorylation and dephosphorylation events. When the hormone binds to the BRI1 receptor, it initiates a process that leads to the inactivation of a negative regulatory kinase called BIN2. This allows the transcription factors BZR1 and BES1 to be dephosphorylated by a phosphatase. Once dephosphorylated, these factors can activate target genes.

**182.** (A) Ethylene acts exclusively as an inhibitor of senescence.
Phytohormones coordinate the plant's response to various stressors. ABA is vital for drought tolerance through the PYR/PYL/RCAR receptors. Jasmonates are essential for defense against insects and necrotrophic pathogens, while salicylic acid mediates resistance against biotrophic pathogens. However, ethylene is well-known for its role in promoting, rather than inhibiting, the senescence of leaves and flowers, and fruit ripening.

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**183.** (D) Hydrogen peroxide and oxygen
Superoxide dismutase is an essential antioxidant enzyme that provides the first line of defense against reactive oxygen species. It catalyzes the dismutation of the highly reactive superoxide radical into two less toxic products: hydrogen peroxide and molecular oxygen. While hydrogen peroxide is still reactive, it can be further detoxified by other enzymes like catalase, preventing the formation of more harmful radicals.

**184.** (B) Molybdenum
Superoxide dismutase enzymes are categorized based on the metal cofactor present in their active site. Plants typically possess three distinct types: copper/zinc-SOD found in the cytosol and chloroplasts, manganese-SOD located in the mitochondria and peroxisomes, and iron-SOD found in the chloroplasts of some species. Molybdenum is not a cofactor for SOD, but it is essential for nitrate reductase.

**185.** (A) $H_2O_2$ is reduced by Ascorbate Peroxidase → Monodehydroascorbate is regenerated →

Glutathione is oxidized → Glutathione Reductase reduces GSSG
The Halliwell-Asada cycle is a crucial pathway for detoxifying hydrogen peroxide, especially in chloroplasts. First, ascorbate peroxidase uses ascorbate to reduce $H_2O_2$ to water. The resulting monodehydroascorbate is then regenerated back to ascorbate, often using reduced glutathione. This process oxidizes glutathione to GSSG. Finally, glutathione reductase uses NADPH to reduce GSSG back to GSH, completing the maintenance of antioxidants.

**186.** (D) Peroxisome
Catalase is a highly efficient heme-containing enzyme that breaks down hydrogen peroxide into water and oxygen. In plant cells, it is primarily localized within the peroxisomes. This localization is significant because the peroxisome is a major site of $H_2O_2$ production during processes like photorespiration and the β-oxidation of fatty acids. By sequestering catalase, the cell can neutralise hydrogen peroxide rapidly.

**187.** (A) Lignin biosynthesis and cell wall cross-linking
Guaiacol peroxidases are a large family of heme enzymes in plants that utilize hydrogen peroxide to oxidize various substrates. They are primarily found in the cell wall and vacuole. Their biological roles include the polymerization of lignin precursors and the cross-linking of cell wall components like extensins and pectins. These activities are essential for the structural integrity of the plant.

**188.** (C) Hydrogen peroxide
Ascorbate peroxidase is a key enzyme in the antioxidant defense system of plants, particularly within the chloroplasts. Its function is to reduce hydrogen peroxide to water. To achieve this, APX uses ascorbic acid as a specific and necessary electron donor. This reaction produces monodehydroascorbate radicals, which must then be recycled back to ascorbate to ensure the continued detoxification of peroxide.

**189.** (A) NADPH
Glutathione reductase is the enzyme responsible for maintaining the cellular supply of reduced glutathione. It catalyzes the reduction of oxidized glutathione back to its monomeric form. This reaction requires the input of high-energy electrons, which are strictly provided by the coenzyme NADPH. By keeping the GSH/GSSG ratio high, the enzyme ensures that glutathione is available for antioxidant defense and the regeneration of ascorbate.

**190.** (A) Preventing cellular damage from metabolic and stress-induced ROS.
Plants produce reactive oxygen species as normal byproducts of photosynthesis and respiration. Under stressful conditions, the production of these radicals can increase significantly, leading to oxidative damage of lipids, proteins, and DNA. Antioxidant defense systems, comprising both enzymatic and non-enzymatic components, are necessary to scavenge these ROS and maintain redox homeostasis. This protection is vital for plant survival.

**191.** (C) Reduced glutathione (GSH)
Dehydroascorbate reductase is an essential enzyme in the ascorbate-glutathione cycle. When ascorbate is oxidized to dehydroascorbate during the detoxification of hydrogen peroxide, DHAR facilitates its regeneration. The enzyme uses reduced glutathione as an electron donor to convert dehydroascorbate back into its active form, ascorbic acid. This reaction link ensures that the pool of ascorbate is maintained for continuous protection.

**192.** (D) Hydroxyl radical ($^{\cdot}OH$)
The hydroxyl radical is the most reactive and toxic species among the reactive oxygen species. It is produced from hydrogen peroxide in the presence of transition metals like iron via the Fenton reaction. Because of its extreme reactivity, it can indiscriminately damage any biological molecule it encounters. There are no known enzymatic scavenging systems to neutralize it, making it exceptionally dangerous.

**193.** (A) It exclusively localized in the cytosol.
The ascorbate-glutathione cycle is a vital pathway for managing oxidative stress and is found in several parts of the plant cell. While it is present in the cytosol, it is also highly active in the chloroplasts, mitochondria, and peroxisomes. The pathway uses NADPH to drive the cyclic regeneration of ascorbate and glutathione, protecting the photosynthetic apparatus from damage.

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**194.** (B) Quenching triplet chlorophyll and singlet oxygen
In addition to their role as accessory light-harvesting pigments, carotenoids are essential for photoprotection in the chloroplast. During high light intensity, they quench the energy from excited triplet-state chlorophyll molecules, which would otherwise react with oxygen to form harmful singlet oxygen. Carotenoids can also directly quench singlet oxygen themselves. This mechanism prevents the destruction of the photosynthetic reaction centers.

**195.** (D) Plastids (chloroplast membranes)
Tocopherols are fat-soluble antioxidants that are uniquely synthesized by photosynthetic organisms. Their biosynthetic pathway is located within the plastids. Due to their highly lipophilic nature, tocopherols are primarily found within the thylakoid membranes of chloroplasts. There, they play a critical role in protecting the membrane lipids from peroxidation by scavenging singlet oxygen and lipid peroxyl radicals generated during light reactions.

**196.** (A) Polyhydroxylated phenolic rings.
Flavonoids are a diverse class of plant secondary metabolites with strong antioxidant properties. Their effectiveness as scavengers of reactive oxygen species is primarily due to their chemical structure, which consists of two phenolic rings connected by a heterocyclic ring. The presence of multiple hydroxyl groups on these rings allows them to easily donate electrons to neutralize free radicals and stabilize molecules.

**197.** (C) Superoxide and singlet oxygen
While ascorbic acid acts as a co-factor for many enzymes, it also functions as a potent non-enzymatic antioxidant. It can directly react with and neutralize several reactive oxygen species, including superoxide, singlet oxygen, and the hydroxyl radical. By donating electrons without the need for a catalytic protein, ascorbate provides a versatile and immediate defense against oxidative stress throughout the plant.

**198.** (A) Carotenoids
B-carotene is a well-known member of the carotenoid family, which are tetraterpene pigments synthesized by plants. It consists of a long chain of conjugated double bonds with an ionone ring at both ends. This structure allows it to absorb light in the blue-green spectrum and perform critical antioxidant functions. As a provitamin A precursor, it is also highly significant.

**199.** (D) Chromanol ring
The molecular structure of tocopherol consists of a long, hydrophobic phytyl side chain and a hydrophilic head group known as the chromanol ring. The antioxidant activity is entirely localized in the chromanol ring, which contains a hydroxyl group. This group can donate a hydrogen atom to neutralize free radicals. The resulting tocopheroxyl radical can then be regenerated by other antioxidant molecules.

**200.** (A) Both A and R are true and R is the correct explanation of A.
Anthocyanins are water-soluble pigments that produce red, purple, and blue colors in many plant organs. Their accumulation is often induced by environmental stressors like high light or low temperatures. In these conditions, anthocyanins act as photoprotectants by absorbing excess light and shielding the underlying tissues. Furthermore, they are located in the vacuole, where they can effectively scavenge oxygen radicals.

**201.** (C) Flavonols
Flavonols are a specific class of flavonoids that play a key role in protecting plants from solar radiation. They possess an absorption spectrum that specifically targets the ultraviolet-B range. By accumulating in the epidermal layers of leaves, flavonols act as a sunscreen that filters out harmful UV light before it can reach the sensitive photosynthetic tissues and the nuclear DNA.

**202.** (D) Violaxanthin, antheraxanthin, and zeaxanthin.
The xanthophyll cycle is an essential photoprotective mechanism that helps dissipate excess light energy as heat. Under high light intensity, the pigment violaxanthin is converted into zeaxanthin through the intermediate antheraxanthin. Zeaxanthin is a potent quencher that facilitates non-photochemical quenching. When light levels decrease, the process is reversed. This reversible interconversion allows the plant to adapt to rapidly changing conditions.

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**203.** (A) Ascorbic acid
The apoplast, which is the space outside the cell membrane, is often the first site of contact for environmental pollutants like ozone. Ascorbic acid is the primary water-soluble antioxidant present in this compartment. By maintaining a pool of reduced ascorbate in the apoplast, the plant can effectively neutralize ozone and its derivatives before they can cause damage to the membrane.

**204.** (B) They only operate in the dark.
Non-enzymatic antioxidants like ascorbate, glutathione, carotenoids, and tocopherols are essential for plant health and operate continuously. They are particularly important during the day when light-driven reactions in the chloroplast generate significant amounts of reactive oxygen species. These molecules often work in tandem with enzymatic systems to provide a robust defense against oxidative stress regardless of the time of day.

**205.** (B) B-carotene
Provitamin A refers to certain carotenoid pigments that can be converted into retinol within the animal body. B-carotene is the most potent and abundant of these provitamin compounds synthesized by plants. It consists of two retinyl groups and is found in high concentrations in orange-colored vegetables and dark leafy greens. For many, consuming β-carotene is the primary way to meet Vitamin A requirements.

**206.** (A) Isopentenyl pyrophosphate (IPP) and Dimethylallyl pyrophosphate (DMAPP)
The biosynthesis of all terpenoids, including sterols, starts with the five-carbon building blocks isopentenyl pyrophosphate and its isomer, dimethylallyl pyrophosphate. In plants, these units are combined to form larger intermediates like farnesyl pyrophosphate. Two FPP molecules then condense to form squalene, a thirty-carbon hydrocarbon. Squalene is subsequently oxidized and cyclized to produce various sterol precursors, such as cycloartenol.

**207.** (C) Tyrosine
Alkaloids are a diverse group of nitrogen-containing secondary metabolites, many of which have significant pharmacological activities. Morphine, codeine, and other benzylisoquinoline alkaloids are synthesized in the opium poppy through a complex pathway. This pathway begins with the amino acid tyrosine. Tyrosine is first converted into various intermediates which then condense to form the characteristic ring structure of this diverse alkaloid class.

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**208.** (D) Oxaloacetate / Aspartate.
Methionine is an essential sulfur-containing amino acid synthesized in plants via the aspartate pathway. The carbon skeleton is derived from oxaloacetate, an intermediate of the citric acid cycle. Oxaloacetate is first converted to aspartate, which then undergoes a series of reactions to form homoserine. Subsequent steps involve the addition of sulfur and methyl groups to finally produce the methionine molecule.

**209.** (C) Shikimate / Phenylpropanoid pathway
Lignin is a major structural component of the secondary cell walls of vascular plants, providing rigidity and hydrophobicity. Its synthesis starts with the_shikimate pathway, which produces the aromatic amino acid phenylalanine. Phenylalanine is then processed through the phenylpropanoid pathway to form various monolignols. These precursors are exported to the cell wall, where they undergo oxidative polymerization to form lignin.

**210.** (A) P-II, Q-IV, R-I, S-III
Tryptophan is the primary precursor for the synthesis of the auxin indole-3-acetic acid. Phenylalanine serves as the starting point for the phenylpropanoid pathway, leading to flavonoids and lignin. Squalene is the direct thirty-carbon precursor for phytosterols. Finally, geranylgeranyl pyrophosphate is the twenty-carbon building block used to produce carotenoids and the phytyl side chain of chlorophyll in plants.

**211.** (D) Heme and Chlorophyll
In plants, the synthesis of porphyrins is essential for energy metabolism. The pathway begins with the conversion of the amino acid glutamate into 5-aminolevulinic acid. Eight molecules of ALA are then combined and modified to form protoporphyrin IX. Depending on the metal ion inserted into the center of the ring, this common precursor leads to the formation of heme or chlorophyll.

**212.** (A) Vitamin D2
Ergosterol is a sterol found in fungi and some plants that serves a similar structural role to cholesterol. When exposed to ultraviolet

radiation, ergosterol undergoes a photochemical reaction that converts it into ergocalciferol, also known as Vitamin D2. This makes ergosterol a provitamin form of Vitamin D. While animals primarily utilize Vitamin D3, Vitamin D2 is an important dietary source.

**213.** (C) Arginine and Ornithine
Polyamines are small, positively charged organic molecules involved in cell growth, development, and stress tolerance. In plants, their biosynthesis primarily utilizes the amino acids arginine and ornithine. These amino acids are decarboxylated to produce putrescine, the simplest polyamine. Putrescine then serves as the substrate for the synthesis of larger polyamines like spermidine and spermine. This pathway responds rapidly to stimuli.

**214.** (A) Pentose Phosphate Pathway
The shikimate pathway, which produces aromatic amino acids and secondary metabolites like lignin, requires two starting materials. One is phosphoenolpyruvate from glycolysis. The other is erythrose-4-phosphate, a four-carbon sugar intermediate. This molecule is uniquely generated during the non-oxidative phase of the pentose phosphate pathway through the activity of the enzyme transaldolase. The availability of E4P is often a limiting factor.

**215.** (D) MEP (non-mevalonate) pathway
Chlorophyll molecules consist of a light-absorbing porphyrin head and a long, hydrophobic phytyl tail. While the head is derived from glutamate, the phytyl tail is a twenty-carbon isoprenoid. In plants, this side chain is synthesized primarily through the methylerythritol phosphate pathway located within the plastids. The MEP pathway produces geranylgeranyl pyrophosphate, which is subsequently reduced before being attached to chlorophyllide.

**216.** (B) Assisting in protein folding and preventing aggregation.
Heat Shock Proteins are a conserved group of proteins whose expression increases under stress. They function as molecular chaperones, ensuring the proper folding of newly synthesized polypeptides and helping refold proteins that have been denatured by heat. By binding to exposed hydrophobic regions, HSPs prevent damaged proteins from forming insoluble aggregates, which could otherwise disrupt normal physiological functions of the cell.

**217.** (C) Hsp70
The Hsp70 family is one of the most ubiquitous and essential classes of molecular chaperones. These proteins use the energy from ATP hydrolysis to bind and release nascent polypeptide chains as they emerge from the ribosome. This transient binding prevents premature folding or inappropriate interactions. Hsp70 also plays a critical role in transporting proteins across membranes into organelles like the chloroplasts.

**218.** (D) Function independently of ATP binding and hydrolysis.
Small Heat Shock Proteins are a diverse group of chaperones characterized by a conserved α-crystallin domain. Unlike the Hsp70 and Hsp90 families, sHSPs do not possess an ATPase activity and therefore function independently of ATP. Instead, they act as molecular sponges that sequester misfolded proteins during stress. This prevents aggregation until the stress subsides and ATP-dependent chaperones can assist.

**219.** (A) Disaggregate and resolubilize already aggregated protein complexes.
While many chaperones prevent protein aggregation, the Hsp100 family is uniquely capable of reversing it. These ATP-driven machines act as unfoldases that can pull individual polypeptide chains out of established protein aggregates. Once the protein is extracted and unfolded, it can then be refolded by other chaperone systems like Hsp70. This disaggregation activity is essential for recovery following extreme heat stress.

**220.** (B) Hsp90
Hsp90 is a highly specialized molecular chaperone that primarily interacts with client proteins that are already near their native conformation. It is particularly known for its role in maintaining the stability and activity of various signaling molecules, including steroid hormone receptors. By regulating these key proteins, Hsp90 acts as a central hub that integrates environmental signals with the control of growth.

**221.** (B) Heat Shock Factors (HSFs)
The rapid induction of Heat Shock Protein genes is controlled by a family of specialized transcription factors called Heat Shock Factors.

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Under normal conditions, HSFs are kept in an inactive state through their interaction with specific chaperones. Upon heat stress, the presence of misfolded proteins titrates away the chaperones, allowing the HSFs to trimerize and bind to conserved promoter elements.

**222.** (A) Random, unregulated protein aggregation.
Proteostasis is the integrated biological network that maintains the health and functionality of the cellular proteome. It includes regulated processes such as protein synthesis, chaperone-mediated folding, and the targeted degradation of damaged proteins. These systems work together to prevent the buildup of toxic proteins. In contrast, unregulated protein aggregation is a sign of proteostasis failure, occurring when defense mechanisms are overwhelmed.

**223.** (C) Providing a secluded environment for protein folding.
Hsp60 proteins, often called chaperonins, form large barrel-shaped oligomeric complexes that act as folding cages. A misfolded protein enters the central cavity of the barrel, which is then capped by a co-chaperone. This sequestered environment prevents the protein from interacting with other molecules in the crowded cytoplasm, allowing it to fold in isolation. The release is triggered by ATP.

**224.** (D) They function as ATP-driven unfoldases.
Plant small Heat Shock Proteins are remarkably abundant and diverse, containing a characteristic α-crystallin domain. They typically assemble into large, multi-subunit oligomers that sequester denatured proteins during stress. Importantly, sHSPs are distinct from larger chaperones because they do not utilize ATP to perform their function. Instead of actively refolding proteins, they act as passive holders that prevent irreversible aggregation.

**225.** (C) ATP hydrolysis
The chaperone cycle of Hsp70 is strictly governed by its ability to bind and hydrolyze ATP. The protein consists of two domains: a nucleotide-binding domain and a substrate-binding domain. When ATP is bound, the chaperone has a low affinity for substrates. The hydrolysis of ATP induces a conformational change that causes the chaperone to bind tightly to the unfolded protein for refolding.

**226.** (B) Exposed hydrophobic residues.
In their native, correctly folded state, proteins typically bury their non-polar, hydrophobic amino acid residues within the interior core. When a protein becomes denatured due to stress or is in a nascent state, these hydrophobic regions become exposed to the aqueous environment. Molecular chaperones recognize and bind to these patches, preventing them from interacting inappropriately with other proteins and forming aggregates.

**227.** (D) Degradation by the 26S proteasome.
When a protein is so severely damaged that it cannot be refolded by the chaperone network, the cell must remove it. These irreparable proteins are typically tagged with ubiquitin through a specific enzymatic cascade. The polyubiquitinated protein is then recognized and degraded by the 26S proteasome, a large protease complex. This process ensures that the building blocks are recycled efficiently.

NODIA

**228.** (A) Replicate its nucleic acids or translate its proteins.
Viruses are obligate intracellular parasites that rely on the host's machinery to survive. To defend against viral infections, plants produce specialized compounds that target the fundamental stages of the viral life cycle. These substances may inhibit the replication of the viral genome or block the translation of viral proteins. By interfering with these essential processes, the plant can effectively limit viral spread.

**229.** (C) Halting translation by cleaving a 28S rRNA adenine residue.
Ribosome-inactivating proteins are potent plant toxins that exhibit a highly specific enzymatic activity. They act as N-glycosidases that target the large subunit of the ribosome. Specifically, they cleave a single, conserved adenine residue from the 28S ribosomal RNA. This irreversible modification prevents the binding of elongation factors, thereby completely halting protein synthesis and leading to the death of cells.

**230.** (A) Specifically induced in response to pathogen attack or related stress.
Pathogenesis-related proteins are a diverse group of plant proteins that are normally present at low levels but are rapidly synthesized upon infection. Their induction is a part of the plant's active defense response and can also be triggered

by various abiotic stressors. PR proteins possess various antimicrobial activities, such as degrading fungal cell walls, contributing significantly to both local and systemic immunity.

**231.** (D) PR-3
The classification of pathogenesis-related proteins is based on their structural and functional characteristics. The PR-3 family specifically consists of chitinases. Chitin is a major component of fungal cell walls but is absent in plants. By producing PR-3 proteins, the plant can enzymatically degrade the chitin in the invading fungus, which compromises its structural integrity. This makes chitinases a powerful tool.

**232.** (B) Thaumatin-like proteins
The PR-5 family of pathogenesis-related proteins is commonly known as thaumatin-like proteins because of their significant sequence similarity to thaumatin. These proteins are involved in defense against fungal pathogens. They are believed to function by permeabilizing the fungal cell membrane, leading to the leakage of intracellular contents and subsequent death of the pathogen. This activity is a key component of immunity.

**233.** (C) Systemic acquired resistance (SAR).
When a plant successfully recognizes a pathogen, it often develops a systemic defense response. This involves the long-distance transport of signals that trigger the expression of pathogenesis-related genes in tissues far from the initial site of infection. This phenomenon provides the entire plant with enhanced protection against a broad spectrum of future pathogens. The accumulation of PR proteins marks this immunity.

**234.** (D) Type 1 Ribosome-inactivating protein (RIP)
The Pokeweed antiviral protein is a classic example of a Type 1 Ribosome-inactivating protein. Type 1 RIPs consist of a single polypeptide chain that possesses the specific N-glycosidase activity required to inactivate ribosomes. Unlike Type 2 RIPs, they lack a lectin B-chain, which makes them less toxic to intact animal cells. Within the plant, PAP provides defense against viral infections.

**235.** (A) Fungi.
Plants often deploy multiple defensive enzymes simultaneously to achieve a more effective attack against pathogens. B-1,3-glucanases and chitinases are frequently induced together because their substrates, glucans and chitin, are the primary structural components of fungal cell walls. By working synergistically, these enzymes can more efficiently break down the complex fungal wall than either enzyme could alone, providing robust barriers.

**236.** (B) Binding to the pathogen membrane and causing pore formation/leakage.
Defensins and thionins are small, cysteine-rich antimicrobial peptides that are part of the plant's innate immune system. These peptides are typically positively charged, which allows them to interact with the negatively charged phospholipids found in the membranes of fungi and bacteria. Upon binding, they insert into the bilayer and form pores, leading to leakage and the death of the pathogen.

**237.** (A) Salicylic acid
Salicylic acid is a central signaling molecule in the regulation of plant defense responses. It is essential for the induction of a large set of pathogenesis-related genes, including those that encode chitinases and glucanases. Following a pathogen attack, salicylic acid levels rise both locally and systemically. This increase triggers a signaling cascade that culminates in the massive activation of defensive PR genes.

**238.** (A) They target and inactivate host DNA polymerases.
Ribosome-inactivating proteins are specialized enzymes found across many plant species. Their primary action is the highly specific N-glycosidase activity that removes a single adenine from ribosomal RNA, thereby irreversibly stopping protein synthesis. They are categorized into Type I and Type II forms. Crucially, RIPs do not target DNA polymerases; their toxicity is strictly due to their interference with translation.

**239.** (B) An effector binds a non-active site causing conformation change.
Allosteric regulation is a sophisticated mechanism for controlling enzyme activity. It occurs when a small regulatory molecule, called an effector, binds to a specific site on the enzyme that is distinct from the active site. This binding induces a conformational change in the overall protein structure, which can either increase or

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decrease the enzyme's affinity for its substrate, allowing metabolic adjustments.

**240.** (D) The first enzyme is inhibited by the final pathway product.
Feedback inhibition is a common regulatory strategy used by cells to prevent the wasteful overproduction of metabolic products. In this process, the final product of a biosynthetic pathway acts as an allosteric inhibitor of the first committed enzyme in that same pathway. As the product concentration rises, it binds to the regulatory enzyme and slows down the entire pathway to conserve resources.

**241.** (C) Phosphorylation
Covalent modification is a flexible way to regulate enzyme activity by adding or removing a chemical group. The most prevalent type of reversible modification is phosphorylation. This involves the addition of a phosphate group to specific amino acid residues by enzymes called protein kinases. The phosphate group can be later removed by protein phosphatases. This cycle acts as a powerful switch for many enzymes.

**242.** (B) Running opposing pathways simultaneously without futile cycles.
Eukaryotic cells are subdivided into specialized organelles, which allow for the physical separation of different metabolic processes. This compartmentalization is advantageous because it enables the cell to run potentially conflicting pathways at the same time in different locations. By sequestering enzymes and substrates within specific compartments, the cell can avoid futile cycles and more precisely regulate the flow of metabolites.

**243.** (D) Negative allosteric modulator.
In allosteric regulation, modulators are classified based on their effect on enzyme activity. A molecule that binds to the allosteric site and reduces the enzyme's ability to bind its substrate or perform catalysis is known as a negative allosteric modulator. These molecules are essential for feedback inhibition, where they help slow down metabolic pathways when a product is abundant in cells.

**244.** (A) Kinases
The regulation of many cellular proteins is achieved through the process of phosphorylation. The specific enzymes that catalyze the transfer of a high-energy phosphate group from ATP to a target protein are known as protein kinases. This modification often causes a significant change in the protein's shape and function. Kinases are highly specific, often targeting only a few substrates and metabolic pathways.

NODIA

**245.** (C) Glycolysis in the cytosol, TCA cycle in the mitochondria.
The metabolic breakdown of glucose is physically separated between two major cellular compartments. Glycolysis, the initial phase, occurs entirely within the cytosol of the cell. The resulting pyruvate is then transported across the mitochondrial membranes. Once inside the mitochondrial matrix, the pyruvate is oxidized, and its carbons enter the citric acid cycle for the aerobic stage of energy production and biosynthesis.

**246.** (A) Feedback inhibition with cross-activation.
Metabolic pathways are often interconnected to ensure that the cell maintains a balanced supply of different products. In some cases, a high concentration of an end-product will inhibit the first enzyme of its own pathway. Simultaneously, this same product may bind to and activate the first enzyme of a competing pathway. This cross-activation helps redirect metabolic flux toward other needed molecules.

**247.** (D) Irreversible, involving proteolytic cleavage.
While mechanisms like phosphorylation and allosteric regulation are easily reversible, zymogen activation is characteristically irreversible. A zymogen is an inactive precursor of an enzyme that must be chemically modified to become active. This activation occurs through the specific proteolytic cleavage of peptide bonds, which allows the protein to fold into its functional catalytic conformation. This is common for many digestive enzymes.

**248.** (B) A sigmoidal curve.
Unlike simple enzymes that follow Michaelis-Menten kinetics and produce a hyperbolic velocity curve, allosteric enzymes typically show a sigmoidal relationship between substrate concentration and reaction rate. This sigmoidal curve is a result of cooperativity, where the binding of a substrate molecule to one subunit increases the affinity of others. This allows allosteric enzymes to be sensitive to small changes.

**249.** (C) They allow for massive signal amplification.
Regulatory cascades involving sequential covalent modifications, such as the activation of a series of kinases, provide a major physiological benefit: signal amplification. A single active enzyme at the top of the cascade can catalyze the modification of many molecules of the next enzyme. This multiplicative effect allows a very small initial signal to trigger a large and rapid cellular response.

**250.** (D) Cytosol and Chloroplast
Plants must balance the immediate use of fixed carbon with its long-term storage. This is managed by the physical separation of sucrose and starch synthesis. Starch is synthesized and stored exclusively within the chloroplasts of the leaf cells during the day. In contrast, sucrose is synthesized within the cytosol. The flow of carbon between these two compartments is tightly regulated.

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# CHAPTER 03
# Enzymology

2010

**1.** Which of the following best defines a ribozyme?
(A) A purely proteinaceous biological catalyst
(B) A catalytic RNA molecule
(C) A synthetic enzyme analog
(D) An antibody with catalytic properties

2015

**2.** In structural organization, what constitutes a holoenzyme?
(A) An apoenzyme with its cofactor
(B) The purely protein-based portion of the enzyme
(C) An organic prosthetic group without any protein
(D) An enzyme lacking its necessary metal ion component

2002

**3.** Metalloenzymes are distinguished from metal-activated enzymes primarily because metalloenzymes:
(A) Require loosely associated metal ions for activity
(B) Function independently of any metal ion requirements
(C) Hold tightly bound essential metal ions
(D) Utilize only monovalent alkali metal ions like sodium

2008

**4.** An enzyme classified under EC 3 belongs to which major class?
(A) Oxidoreductases
(B) Transferases
(C) Isomerases
(D) Hydrolases

2006

**5.** Identify the EXCEPT regarding the active site microenvironment:
(A) It provides a non-polar environment for binding.
(B) It utilizes specific amino acid chains for catalysis.
(C) It represents the bulk of total enzyme volume.
(D) It stabilizes the high-energy transition state.

NODIA

2001

**6.** Which statement correctly describes the induced-fit model of enzyme-substrate interaction?
(A) The active site remains perfectly rigid during binding.
(B) Binding induces an enzyme conformational change.
(C) The substrate reshapes while the enzyme stays static.
(D) Pre-existing conformations exist in equal equilibrium.

2013

The primary role of binding energy in enzyme catalysis is best described as:
(A) Providing the thermal energy needed for reaction catalysis
(B) Stabilizing the substrate in its specific ground state
(C) Increasing the overall standard free energy of reaction
(D) Lowering activation energy to stabilize transition states

2008

**8.** Identify the INCORRECT statement regarding chemical mechanisms of catalysis:
(A) Electrostatic catalysis uses hydrophobic bonds.
(B) Acid-base catalysis involves partial proton transfer.
(C) Covalent catalysis requires a transient nucleophilic attack.
(D) Metal-ion catalysis can shield negative substrate charges.

2016

**9.** Identify the correct pair regarding Michaelis-Menten kinetics parameters:
(A) $K_m$ - Maximum velocity of the enzyme
(B) $V_{\max}$ - Substrate concentration at half-maximal velocity
(C) $k_{\text{cat}}$ - Turnover number of the enzyme
(D) $\frac{k_{cat}}{K_m}$ - Measure of enzyme concentration

2023

**10.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option:
Assertion (A): The Lineweaver-Burk plot often distorts experimental error at low substrate concentrations.
Reason (R): The Lineweaver-Burk plot uses the double reciprocal of velocity and substrate concentration.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2024

**11.** In a Ping-Pong bi-bi mechanism, which of the following occurs?
(A) Both substrates must bind before any product is released.
(B) Substrates bind randomly and independently to the site.
(C) A ternary complex is formed during the catalytic reaction.
(D) Product release occurs before second substrate binding.

2011

**12.** Which class of reversible inhibition is characterized by binding exclusively to the enzyme-substrate complex?
(A) Competitive inhibition
(B) Uncompetitive inhibition
(C) Non-competitive inhibition
(D) Mixed inhibition

2003

**13.** Which irreversible inhibitors utilize a latent functional group that is activated only upon binding to the target enzyme's active site?
(A) Affinity labels
(B) Group-specific reagents
(C) Suicide substrates
(D) Transition-state analogs

2003

**14.** What is the physical significance of the temperature coefficient ($Q_{\{10\}}$)?
(A) The temperature at which an enzyme completely denatures.
(B) The precise optimal temperature for maximum velocity.
(C) The activation energy measured at a standard 10°C.
(D) The reaction rate increase factor per 10°C rise.

NODIA

2005

**15.** Consider the following statements regarding coupled enzyme assays:
I. The primary reaction must be the rate-limiting step.
II. The auxiliary enzyme is added in large, non-limiting excess.
III. The primary product is converted to a measurable compound.
Which of the above statements is/are correct?
(A) I and III
(B) II
(C) I and II
(D) III

2018

**16.** Specific activity increases during a successful enzyme purification because:
(A) The total enzyme activity strictly increases throughout.
(B) Total protein drops while target activity is retained.
(C) The enzyme molecules become covalently modified in vitro.
(D) Non-specific enzymes are activated by purification buffers.

2012

**17.** The primary disadvantage of chemical cell disruption methods compared to physical methods is:
(A) Extremely high initial equipment and maintenance costs.
(B) Risk of protein denaturation by chemicals.
(C) Excessive heat generation during the disruption process.
(D) Inability to process small sample volumes efficiently.

2015

**18.** Salting-out precipitates proteins primarily by:
(A) Competing for protein hydration water.
(B) Altering pH to the specific isoelectric point.
(C) Denaturing the secondary structure of proteins.
(D) Cleaving peptide bonds via enzymatic hydrolysis.

2014

**19.** Select the correct order of elution in size-exclusion (gel filtration) chromatography for proteins P (15 kDa), Q (120 kDa), and R (60 kDa):
(A) P, R, Q

(B) P, Q, R
(C) Q, R, P
(D) R, Q, P

2021

**20.** Zymography is a specialized electrophoretic technique primarily used to detect:
(A) Nucleic acid contamination
(B) The exact molecular weight of denatured proteins
(C) The isoelectric point of a protein
(D) Specific enzyme activity within a gel matrix

2015

**21.** Which parameter characterizes the degree of cooperativity in allosteric enzymes?
(A) Michaelis constant
(B) Hill coefficient
(C) Turnover number
(D) Catalytic efficiency

2019

**22.** Zymogen activation is best defined as:
(A) Reversible phosphorylation of a protein enzyme.
(B) Allosteric activation by a metabolic effector.
(C) Irreversible cleavage of a precursor.
(D) Ubiquitination of a structurally active enzyme.

2025

**23.** Isoenzymes are distinct enzyme forms that catalyze the same reaction but differ in:
(A) The specific substrate they catalyze.
(B) Their fundamental catalytic mechanisms.
(C) Their specific coenzyme requirements.
(D) Sequence and physical properties.

2018

**24.** Which condition causes an enzyme reaction to follow zero-order kinetics?
(A) Substrate concentration is much higher than $K_m$
(B) Substrate concentration is equal to $K_m$
(C) Substrate concentration is strictly proportional to velocity
(D) Enzyme concentration is zero

2009

**25.** Consider the following statements regarding the structural organization of enzymes:
I. Coenzymes are tightly bound inorganic ions.
II. Prosthetic groups are covalently or strongly bound organic molecules.
III. Apoenzymes exhibit maximum catalytic activity.
IV. Holoenzymes are structurally complete and catalytically active.
Which of the above statements are correct?
(A) II, III and IV
(B) I and III
(C) II and IV
(D) I, II and IV

2005

**26.** Which structural element provides a stable, covalently bonded framework for specific oxidoreductases?
(A) Soluble NADH molecules used as coenzymes.
(B) Tightly bound FAD prosthetic groups.
(C) Free magnesium ions in the local solution.
(D) Exchangeable zinc ions for structural support.

2001

**27.** The most appropriate explanation for metal ions facilitating enzymatic catalysis is that they:
(A) Lower transition state free energy.
(B) Act as strong bases to extract protons.
(C) Denature substrates to expose hidden bonds.
(D) Replace the need for an active site cleft.

2005

**28.** Identify the INCORRECT statement concerning enzyme classification:
(A) EC 1 comprises oxidoreductases.
(B) Ligases belong to EC 6 and catalyze joining of two molecules.
(C) Lyases (EC 4) cleave bonds strictly by hydrolysis.
(D) Transferases (EC 2) move functional groups between molecules.

2017

**29.** Identify the correct pair matching an active site interaction with its typical distance:
(A) Covalent bond - 5 to 10 Angstroms
(B) Van der Waals interaction - 15 Angstroms
(C) Salt bridge - 0.5 Angstroms
(D) Hydrogen bond - 2.5 to 3.5 Angstroms

2018

**30.** Match the inhibition types in List I with their corresponding kinetic effects in List II:
List I: (Inhibition Types)
P. Competitive
Q. Non-competitive
R. Uncompetitive
S. Mixed
List II: (Kinetic Effects)
1. $K_m$ increases, $V_{\max}$ unchanged
2. $K_m$ unchanged, $V_{\max}$ decreases

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3. Both $K_m$ and $V_{max}$ decrease
4. $K_m$ increases, $V_{max}$ decreases

(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-1, R-4, S-3
(C) P-3, Q-4, R-1, S-2
(D) P-1, Q-4, R-2, S-3

2016

**31.** A model suggesting enzymes select a complementary shape from a pre-existing ensemble of states describes:
(A) The traditional Lock-and-Key model.
(B) Conformational Selection model.
(C) The induced-Fit interaction model.
(D) The Transition State formulation.

2014

**32.** In the context of catalysis energetics, activation energy is defined as:
(A) Energy difference between products and reactants.
(B) Standard free energy of the overall reaction.
(C) Energy released upon specific substrate binding.
(D) Energy required to reach the transition state.

2019

**33.** The cleavage of a peptide bond by chymotrypsin, involving a transient acyl-enzyme intermediate, exemplifies:
(A) Covalent catalysis
(B) Purely electrostatic catalysis
(C) Metal-ion catalysis
(D) General acid catalysis

2009

**34.** What is the physical significance of the steady-state assumption in deriving the Michaelis-Menten equation?
(A) Substrate concentration remains completely static.
(B) Product concentration equals substrate amount.
(C) ES formation rate equals its breakdown rate.
(D) The reaction has reached thermodynamic balance.

2009

**35.** Consider the following statements concerning the Eadie-Hofstee plot:
I. It plots velocity vs velocity/substrate concentration.
II. It spreads data points at high substrate concentrations.
III. The y-intercept represents $\frac{V_{max}}{K_m}$.
IV. The slope of the line is strictly positive.
V. It provides a more uniform distribution of errors than Lineweaver-Burk.

Which of the above statements are correct?
(A) I, II, III and IV
(B) I, II and V
(C) II, III and V
(D) I, III, IV and V

2019

**36.** An enzyme exhibits ordered sequential kinetics. This logically infers that:
(A) Any substrate can bind first without restriction.
(B) Product release occurs before second substrate binding.
(C) Substrates do not interact within the active site.
(D) Substrates bind in a specific sequence.

NODIA

2014

**37.** Which advantage does a Dixon plot primarily offer in enzyme kinetics?
(A) It easily visualizes substrate inhibition.
(B) It directly calculates the inhibition constant ($K_i$).
(C) It accurately determines $V_{max}$ without extrapolation.
(D) It plots raw data without linear transformation.

2018

**38.** Diisopropyl fluorophosphate (DIPF) covalently modifies serine residues. Which classification best applies to it?
(A) A reversible type of competitive inhibitor.
(B) A stable transition-state analog molecule.
(C) Irreversible group-specific reagent.
(D) An allosteric activator of enzyme kinetics.

2022

**39.** Identify the INCORRECT statement regarding physical factors affecting kinetics:
(A) Optimal pH favors active site ionization.
(B) Supra-optimal temperature induces denaturation.
(C) Low temperature reduces molecular kinetic energy.
(D) High ionic strength increases solubility.

2015

**40.** A continuous enzyme assay differs from a discontinuous assay because it:
(A) Monitors reaction progress over time.
(B) Requires separating products before measurement.

(C) Uses radioisotopes for tracking reactions.
(D) Measures only the final end-point of reaction.

2018

**41.** One Katal is defined as the amount of enzyme that converts:
(A) 1 micromole of substrate per minute
(B) 1 mole of substrate per second
(C) 1 millimole of substrate per hour
(D) 1 nanomole of product per millisecond

2016

**42.** Sonication disrupts cells through:
(A) Enzymatic digestion of cell wall parts.
(B) Acoustic cavitation shear stress.
(C) Osmotic shock inducing cell lysis effects.
(D) Chemical solubilization of cell membranes.

2010

**43.** Precipitation of proteins at the isoelectric point occurs due to:
(A) Minimum solubility at the isoelectric point.
(B) Maximum solubility at the isoelectric point.
(C) Maximum net charge at the isoelectric point.
(D) High repulsion at the isoelectric point.

2003

**44.** In ion-exchange chromatography using a DEAE matrix at pH 8.0, an enzyme with a pI of 5.0 will:
(A) Carry a positive charge and bind matrix.
(B) Remain neutral and elute in void volume.
(C) Carry a negative charge and bind matrix.
(D) Repel the negatively charged bead matrix.

2003

**45.** Identify the correct procedural sequence for purifying an intracellular enzyme:
I. Affinity chromatography
II. Ammonium sulfate precipitation
III. Cell disruption
IV. Centrifugation to remove debris
(A) III, IV, II, I
(B) III, II, IV, I
(C) IV, III, I, II
(D) II, III, IV, I

2014

**46.** Native-PAGE separates proteins based on:
(A) Mass alone
(B) Mass-to-charge ratio solely
(C) Charge, size, and 3D shape
(D) Isoelectric point strictly

2022

**47.** The Concerted (MWC) model for allosteric regulation stipulates that:
(A) Subunits change conformation sequentially.
(B) All subunits share the same conformation.
(C) Ligands trap subunits without site effects.
(D) Substrates bind exclusively to the T state.

2018

**48.** Phosphorylation of enzymes involves the transfer of a phosphate group primarily to which amino acid residues?
(A) Alanine, Glycine, Valine
(B) Cysteine, Methionine, Tryptophan
(C) Aspartate, Glutamate, Lysine
(D) Serine, Threonine, Tyrosine

2007

**49.** The presence of multiple isoenzymes of Lactate Dehydrogenase (LDH) allows tissues to:
(A) Regulate tissue metabolic requirements.
(B) Utilize different catalytic enzyme mechanisms.
(C) By-pass the need for a specific coenzyme.
(D) Operate efficiently only at high temperatures.

2018

**50.** Abzymes exhibit catalytic activity because they are engineered to bind:
(A) The specific substrate in its ground state
(B) The reaction's transition-state analog
(C) Competitive inhibitors tightly
(D) Metallic cofactors exclusively

2018

**51.** The primary distinction between an apoenzyme and a coenzyme is that:
(A) Apoenzymes require coenzymes for activity.
(B) Apoenzymes are small organic cofactor molecules.
(C) Apoenzymes are always active without cofactors.
(D) They are considered to be synonymous terms.

2019

**52.** Which phenomenon illustrates the role of metal-activated enzymes?
(A) Iron firmly complexed in cytochromes.
(B) Zinc structurally integral to anhydrase.
(C) Copper fixed within tyrosinase enzymes.
(D) Free $Mg^{2+}$ requirement for kinases.

2023

**53.** An enzyme that catalyzes the epimerization of a substrate is classified as a/an:
(A) Ligase
(B) Lyase
(C) Isomerase

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(D) Hydrolase

2011

**54.** Identify the EXCEPT among characteristics of the active site:
(A) It is usually a cleft or crevice.
(B) It forms a microenvironment of polarity.
(C) It prevents conformational shifts.
(D) It binds via weak non-covalent bonds.

2023

**55.** Consider the following statements regarding enzyme-substrate models:
I. The lock-and-key model successfully explains transition state stabilization.
II. The induced-fit model accounts for enzyme flexibility.
III. Conformational selection requires the substrate to force the enzyme into shape.
IV. Enzyme active sites are perfectly complementary to the substrate ground state.
Which of the above statements are INCORRECT?
(A) I, II and IV
(B) I, III and IV
(C) II and III
(D) I and IV

2013

**56.** Transition state stabilization directly results in:
(A) Decreasing the reaction energy barrier.
(B) Lowering standard free energy of products.
(C) Shifting equilibrium to favor the products.
(D) Increasing ground state energy of enzyme.

2014

**57.** Which catalytic mechanism utilizes a nucleophile on the enzyme to form a transient intermediate with the substrate?
(A) General acid catalysis
(B) Covalent catalysis
(C) Metal-ion catalysis
(D) Electrostatic catalysis

2013

**58.** Under Michaelis-Menten assumptions, when the velocity of the reaction is precisely half of Vmax:
(A) $[S] = K_m$
(B) $[S] = 2K_m$
(C) $K_m = V_{\max}$
(D) $[S] = \frac{V_{\max}}{2}$

2024

**59.** The Hanes-Woolf plot transforms kinetic data by graphing:
(A) $\frac{[S]}{v}$ vs $[S]$
(B) $\frac{1}{v}$ vs $\frac{1}{[S]}$
(C) $v$ vs $\frac{v}{[S]}$
(D) $\frac{1}{v}$ vs $[I]$

2001

**60.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option:
Assertion (A): Transaminases generally operate via a Ping-Pong bi-bi kinetic mechanism.
Reason (R): Transaminases transfer an amino group to a bound prosthetic group before the second substrate binds.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**61.** A mixed inhibitor primarily functions by binding to:
(A) Free enzyme only
(B) Enzyme-substrate complex only
(C) An allosteric site strictly competing with the active site
(D) Both free enzyme and enzyme-substrate complex

2011

**62.** Affinity labels differ from group-specific reagents primarily because they:
(A) React with any exposed amino acid groups.
(B) Bind reversibly to the enzyme surface.
(C) Target the active site by resembling the substrate.
(D) Operate only at extreme acidic pH levels.

2008

**63.** A decrease in pH below the optimal range generally affects enzyme activity by:
(A) Cleaving peptide bonds non-specifically.
(B) Altering ionization of active site residues.
(C) Increasing hydrophobic interactions.
(D) Irreversibly precipitating all proteins.

2023

**64.** Which analytical advantage justifies using a coupled enzyme assay?
(A) It eliminates the need for substrate.
(B) Increases specific activity of enzymes.
(C) Works when primary reaction is fastest.
(D) Allows measurement of silent products.

NODIA

2015

**65.** Consider the following statements regarding enzyme purification metrics:

I. Specific activity is total activity divided by total protein.

II. Purification fold decreases as purity increases.

III. Percentage yield compares retained activity to initial total activity.

Which of the above statements are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I

2017

**66.** A non-competitive inhibitor alters parameters such that:

(A) Apparent $K_m$ increases, apparent $V_{\max}$ decreases
(B) Apparent $K_m$ decreases, apparent $V_{\max}$ decreases
(C) Apparent $K_m$ is unchanged, apparent $V_{\max}$ decreases
(D) Apparent $K_m$ increases, apparent $V_{\max}$ is unchanged

2002

**67.** Organic solvent precipitation (e.g., using ethanol) works primarily by:

(A) Lowering the dielectric constant to promote protein attraction.
(B) Increasing the dielectric constant of the solvent medium.
(C) Covalently cross-linking protein molecules in solution.
(D) Saturating the solution with specific types of ions.

2017

**68.** Identify the INCORRECT pair regarding chromatography techniques:

(A) Gel filtration - Separation by size.
(B) Ion-exchange - Separation by charge.
(C) Hydrophobic - Separation by surface hydrophobicity.
(D) Affinity - Separation by solubility.

2011

**69.** Molecular weight determination using SDS-PAGE relies on the principle that SDS:

(A) Confers a uniform positive charge to all proteins.
(B) Leaves disulfide bonds completely intact.
(C) Provides a uniform mass-to-charge ratio.
(D) Separates strictly based on native pI.

2002

**70.** According to the Koshland-Némethy-Filmer (KNF) model for allosteric enzymes:

(A) All subunits transition simultaneously.
(B) Ligands only bind to the T-state form.
(C) Sequential conformation changes occur upon binding.
(D) Cooperativity is mathematically impossible.

2001

**71.** The irreversible activation of trypsinogen to trypsin is an example of:

(A) Reversible covalent modification
(B) Allosteric inhibition
(C) Feedback regulation
(D) Proteolytic activation

2017

**72.** The Pyruvate Dehydrogenase complex is structurally advantageous because it:

(A) Channels intermediates between sites.
(B) Increases the transit time of intermediates.
(C) Prevents covalent catalysis from occurring.
(D) Functions effectively without any coenzyme.

2024

**73.** Which feature distinguishes synzymes from naturally occurring enzymes?

(A) They are comprised strictly of RNA molecules.
(B) They are artificially synthesized catalytic polymers.
(C) They are isolated from extreme thermophiles.
(D) They require high heavy metal concentrations.

2010

**74.** Which coenzyme serves as a mobile carrier of acyl groups?

(A) NAD+
(B) Coenzyme A
(C) Pyridoxal phosphate
(D) Biotin

2003

**75.** Consider the following statements concerning enzyme classes:

I. Kinases are generally classified as transferases.

II. Phosphatases belong to the ligase classification.

III. Peptidases belong to the hydrolase classification.

IV. Decarboxylases are categorized under oxidoreductases.

Which of the above statements are INCORRECT?

(A) I and III

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(B) II, III and IV
(C) I, II and IV
(D) II and IV

2015

**76.** The binding site of an enzyme is responsible for:
(A) Lowering the activation energy solely.
(B) Generating a nucleophile for reaction.
(C) Providing specificity and orientation.
(D) Transferring a phosphate group to E.

2005

**77.** Which inference applies to the induced-fit hypothesis?
(A) Catalysis is independent of alignment.
(B) Small substrates bind tighter than large.
(C) Rigid enzymes are the most efficient.
(D) Active site competence follows substrate binding.

2013

**78.** In transition state theory, enzymes achieve rate enhancement by:
(A) Raising the local microenvironment temperature.
(B) Converting reaction to single high step.
(C) Tight binding of the transition state.
(D) Destabilizing the final reaction products.

2009

**79.** General acid-base catalysis involves:
(A) Strictly hydronium and hydroxyl from water.
(B) Proton transfers via amino acid chains.
(C) Complete electron transfer redox reactions.
(D) Covalent bond formation with metal ions.

2004

**80.** Match the kinetic plots in List I with their characteristics in List II:
List I: (Kinetic Plots)
P. Lineweaver-Burk
Q. Eadie-Hofstee
R. Hanes-Woolf
S. Dixon
List II: (Characteristics)
1. $\frac{1}{v}$ versus $[I]$
2. $\frac{[S]}{v}$ versus $[S]$
3. $\frac{1}{v}$ versus $\frac{1}{[S]}$
4. $v$ versus $\frac{v}{[S]}$

(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-3, Q-1, R-4, S-2
(D) P-2, Q-3, R-1, S-4

2016

**81.** Based on steady-state kinetics, $\frac{k_{cat}}{K_m}$ is a measure of catalytic efficiency because it:
(A) Reflects the rate at saturating substrate.
(B) Is strictly determined by enzyme amount.
(C) Ignores the breakdown of the ES complex.
(D) Reflects both catalysis and affinity.

2018

**82.** In an ordered sequential bi-bi reaction:
(A) Lineweaver-Burk plots show parallel lines upon varying the second substrate.
(B) A specific ternary complex forms before any products are released.
(C) Products are released alternately.
(D) The initial velocity is independent of the second substrate.

2014

**83.** Which diagnostic plot characteristic identifies uncompetitive inhibition?
(A) Parallel lines on a Lineweaver-Burk plot
(B) Intersecting lines on the y-axis of a Lineweaver-Burk plot
(C) Intersecting lines on the negative x-axis
(D) Non-linear curves on a Hanes-Woolf plot

2018

**84.** A suicide inhibitor relies on:
(A) Binding weakly to the allosteric site.
(B) Denaturing the enzyme's secondary structure.
(C) Reversibly outcompeting the substrate.
(D) Activation by the enzyme's catalytic mechanism.

NODIA

2002

**85.** Consider the following statements regarding optimal pH effects:
I. Activity strictly increases as pH rises.
II. Extreme pH alters the protonation state of catalytic residues.
III. Substrate ionization may also change with pH.
IV. Enzymes typically maintain a broad, flat pH optimum spanning 6 units.
V. Drastic pH changes can lead to irreversible denaturation.
Which of the above statements are correct?
(A) I, II, III and V
(B) II, III and V
(C) I, III, IV and V
(D) II, IV and V

2005

**86.** The main advantage of a discontinuous enzyme assay is:
(A) It requires fewer total reagents per assay.
(B) It maps out precise velocities in real-time.
(C) It handles reactions without optical changes.
(D) It inherently prevents product inhibition.

2012

**87.** A protein extraction procedure reports a purification fold of 50. This indicates that:
(A) 50% of the initial enzyme activity is lost.
(B) Total protein increased exactly 50-fold.
(C) The final preparation is 100% pure.
(D) Specific activity increased 50-fold.

2018

**88.** Enzymatic cell disruption, using agents like lysozyme, targets:
(A) Bacterial peptidoglycan cell walls.
(B) The phospholipid bilayer of cells.
(C) Intracellular nuclear membranes.
(D) Extracellular protein matrices.

2007

**89.** Ammonium sulfate is preferred for precipitation because of its:
(A) High chaotropic nature which denatures protein.
(B) Ability to decrease the surface tension of water.
(C) High solubility and stabilization of proteins.
(D) Ability to selectively precipitate nucleic acids.

2003

**90.** Hydrophobic interaction chromatography (HIC) typically requires loading the protein sample under which condition?
(A) High salt to enhance interactions.
(B) Low salt concentration to prevent precipitation.
(C) Highly acidic pH to neutralize residues.
(D) Presence of strong detergents like SDS.

2018

**91.** Isoelectric focusing resolves proteins on the basis of their:
(A) Isoelectric point in a pH gradient
(B) Native molecular weight
(C) Overall amino acid sequence length
(D) Hydrophobicity index

2015

**92.** The sigmoidal kinetics exhibited by allosteric enzymes deviate from:
(A) Zero-order kinetics at high substrate concentration
(B) The standard hyperbolic Michaelis-Menten curve
(C) The first-order dependence on enzyme concentration
(D) The steady-state assumption entirely

2021

**93.** A phosphorylation cascade amplifies a biological signal because:
(A) Phosphate groups contain high energy.
(B) One kinase activates many downstream enzymes.
(C) It irreversibly consumes cellular ATP.
(D) It destroys the initial signal molecule.

2004

**94.** The Fatty Acid Synthase multienzyme complex integrates multiple catalytic activities on a single polypeptide chain. This architectural design:
(A) Decreases local solute concentration.
(B) Increases the likelihood of degradation.
(C) Improves efficiency via substrate channeling.
(D) Makes the complex immune to allosteric effects.

2014

**95.** Identify the correct chronological order for the following enzyme kinetic steps in a single-substrate reaction:
I. Formation of enzyme-substrate complex
II. Product release
III. Substrate diffusion to the active site
IV. Formation of enzyme-product complex
(A) III, I, IV, II
(B) I, III, IV, II
(C) III, IV, I, II
(D) I, IV, II, III

2023

**96.** Which unique feature defines an abzyme's development?
(A) Raising antibodies against transition-state analogs.
(B) Randomly mutating specific RNA sequences.
(C) Synthesizing sites on polymer scaffolds.
(D) Harvesting extreme thermophile proteins.

2009

**97.** Identify the correct statement regarding prosthetic groups:
(A) They are tightly bound to the enzyme.
(B) They dissociate during each catalytic cycle.
(C) They are loosely bound organic molecules.
(D) They consist exclusively of metal ions.

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2013

**98.** Metal ions in metalloenzymes can act as electrophiles, meaning they:
(A) Donate electrons to the substrate.
(B) Withdraw electron density to facilitate bond breaking.
(C) Directly donate protons as an acid.
(D) Repel nucleophilic attacks on the enzyme.

2012

**99.** Which enzyme class catalyzes reactions involving geometric or structural changes within a single molecule?
(A) EC 5 (Isomerases)
(B) EC 3 (Hydrolases)
(C) EC 4 (Lyases)
(D) EC 6 (Ligases)

2004

**100.** In an enzyme active site, which amino acid is most commonly involved in acid-base catalysis due to its pKa being near physiological pH?
(A) Alanine
(B) Leucine
(C) Phenylalanine
(D) Histidine

2012

**101.** The concept that enzyme conformation is constantly fluctuating and the substrate binds to a specific active conformation is known as:
(A) The rigid template model
(B) Conformational selection
(C) The concerted allosteric model
(D) The lock-and-key model

2015

**102.** What primarily drives the stabilization of the transition state during enzyme catalysis?
(A) Provision of high thermal kinetic energy.
(B) Covalent modification of final products.
(C) Binding energy from weak interactions.
(D) Irreversible denaturation of the active site.

2003

**103.** Which mechanism involves an enzyme residue acting as an electron-pair donor?
(A) General base catalysis
(B) Metal-ion catalysis
(C) Conformational catalysis
(D) Covalent catalysis

2015

**104.** The value of $K_m$ is an approximate measure of substrate affinity when:
(A) Dissociation rate exceeds catalytic rate.
(B) Catalytic rate exceeds dissociation rate.
(C) The enzyme is fully substrate saturated.
(D) The reaction is essentially irreversible.

2001

**105.** Consider the following criteria for establishing protein purity:
I. A single band on an SDS-PAGE gel.
II. A decreasing specific activity.
III. A single symmetric peak in analytical size-exclusion chromatography.
Which of the above criteria are correct?
(A) I and III
(B) II and III
(C) I and II
(D) I

2021

**106.** In an uncompetitive inhibition Lineweaver-Burk plot, why are the lines parallel?
(A) The inhibitor affects $K_m$ and $V_{\max}$ to the exact same relative extent.
(B) The inhibitor only affects $V_{\max}$.
(C) The inhibitor strictly binds the free enzyme.
(D) The inhibitor irreversibly destroys the enzyme.

2021

**107.** Which method represents a physical technique for cell disruption using high pressure?
(A) French press
(B) Osmotic shock
(C) Detergent lysis
(D) Enzymatic digestion

2016

**108.** Transition-state analogs are potent inhibitors because they:
(A) Irreversibly modify the enzyme covalently.
(B) Rapidly degrade the target enzyme.
(C) Exploit maximal binding energy.
(D) Bind nonspecifically to all residues.

2005

**109.** If a reaction's rate doubles when the temperature increases from 20°C to 30°C, the $Q_{\{10\}}$ value is:
(A) 0.5
(B) 1.0
(C) 2.0
(D) 10.0

2021

**110.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option:
Assertion (A): High ionic strength commonly decreases the solubility of proteins, leading to

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precipitation.
Reason (R): Excess salt ions compete with the protein for surrounding water molecules.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2004

**111.** When calculating enzyme yield, one determines the ratio of:
(A) Final specific activity to initial specific activity
(B) Final total protein concentration to initial total protein concentration
(C) Total mass to total volume
(D) Final total activity to initial total activity

2010

**112.** Why is affinity chromatography often the most selective purification step?
(A) It relies on bulk properties like charge.
(B) It separates by massive size differences.
(C) It employs harsh isolating denaturants.
(D) It uses specific biological ligand interactions.

2005

**113.** Dialysis is used in enzyme purification protocols for:
(A) Salt removal and buffer exchange.
(B) Precipitating target proteins specifically.
(C) Cleaving unwanted active protein fragments.
(D) Determining the protein molecular weight.

2011

**114.** Zymography is performed by copolymerizing a substrate within which matrix?
(A) Agarose for Southern blotting
(B) Silica gel for thin layer chromatography
(C) Polyacrylamide gel for electrophoresis
(D) Cellulose acetate for paper electrophoresis

2008

**115.** Consider the following statements concerning allosteric regulation:
I. Effectors typically bind at the active site.
II. Binding of an effector induces conformational changes.
III. Kinetics often display sigmoidal $v$ vs $[S]$ plots.
Which of the above statements are correct?
(A) I and II
(B) II and III
(C) I and III
(D) III

2011

**116.** A regulatory cascade utilizing sequential zymogen activation is advantageous because it:
(A) Guarantees the reaction is fully reversible.
(B) Eliminates the need for genetic transcription.
(C) Slows down metabolic protein responses.
(D) Provides signal amplification and control.

2021

**117.** Lactate Dehydrogenase (LDH) exists as a tetramer comprising H (heart) and M (muscle) subunits. How many total distinct isoenzyme forms of LDH are possible?
(A) 3
(B) 4
(C) 5
(D) 6

2011

**118.** Which statement is an INCORRECT description of enzyme nature?
(A) Ribozymes use RNA structures to catalyze reactions.
(B) Synzymes are designed by chemists to mimic catalysis.
(C) All enzymes are exclusively made of amino acids.
(D) Holoenzymes contain a protein portion and a non-protein cofactor.

2010

**119.** Which organic molecule functions as a coenzyme in transamination reactions?
(A) Thiamine pyrophosphate (TPP)
(B) Flavin adenine dinucleotide (FAD)
(C) Nicotinamide adenine dinucleotide (NAD+)
(D) Pyridoxal phosphate (PLP)

2021

**120.** Identify the correct pair connecting a metal ion with its physiological enzyme:
(A) Zinc - Carbonic anhydrase
(B) Iron - Hexokinase
(C) Magnesium - Cytochrome oxidase
(D) Calcium - Pyruvate kinase

2022

**121.** Enzyme Subclass EC 3.4 predominantly targets which type of chemical bond?
(A) Glycosidic bonds
(B) Peptide bonds
(C) Ester bonds
(D) Phosphodiester bonds

NODIA

2019

**122.** The specificity pocket in the active site of chymotrypsin primarily accommodates:
(A) Small aliphatic side chains
(B) Bulky aromatic side chains
(C) Negatively charged side chains
(D) Positively charged side chains

2022

**123.** The fundamental flaw in the rigid lock-and-key model is that it fails to explain:
(A) Transition state stabilization.
(B) The precision of enzyme specificity.
(C) Physical binding of specific substrates.
(D) The existence of competitive inhibitors.

2021

**124.** The difference between the free energy of the transition state and the free energy of the ground state reactants defines the:
(A) Standard free energy of reaction ($\Delta G^{\circ}$)
(B) Enthalpy of reaction ($\Delta H$)
(C) Activation energy ($\Delta G^{\dagger}$)
(D) Equilibrium constant ($K_{eq}$)

2010

**125.** Consider the following statements regarding enzyme inhibition mechanisms:
I. Competitive inhibitors structurally resemble the substrate.
II. Non-competitive inhibitors prevent substrate binding completely.
III. Suicide inhibitors undergo partial catalytic processing.
IV. Uncompetitive inhibitors bind the free enzyme.
Which of the above statements are INCORRECT?
(A) I and III
(B) II and IV
(C) I, II and IV
(D) II, III and IV

2013

**126.** A Schiff base formation during an enzymatic reaction is a classic intermediate of:
(A) Electrostatic catalysis
(B) Metal-ion catalysis
(C) Covalent catalysis
(D) Allosteric regulation

2021

**127.** Which assumption is fundamental to deriving the Michaelis-Menten equation?
(A) The reaction is totally irreversible globally.
(B) Initial product concentration is zero.
(C) Enzyme amount exceeds substrate concentration.
(D) The ES complex immediately decomposes to E and S.

2014

**128.** In a Dixon plot ($\frac{1}{v}$ vs $[I]$) for a competitive inhibitor, lines at different substrate concentrations will intersect:
(A) Precisely on the vertical y-axis.
(B) In the third quadrant at $x = -K_m$.
(C) On the horizontal x-axis at zero.
(D) In the second quadrant at $-K_i$.

2024

**129.** Cleland's notation is standardly used to represent:
(A) Multi-substrate kinetic mechanisms.
(B) Thermodynamic folding pathways of proteins.
(C) Atomic distances within active site clefts.
(D) Evolutionary divergence of isoenzyme forms.

2018

**130.** Match the irreversible inhibitor classes in List I with their corresponding actions in List II:
List I: (Inhibitor Class)
P. Affinity label
Q. Group-specific reagent
R. Suicide substrate
S. Transition-state analog
List II: (Action)
1. Reacts with specific R-groups universally
2. Mechanism-based inactivation
3. Mimics substrate tightly, permanently blocking site
4. Resembles substrate but possesses a reactive group
(A) P-4, Q-1, R-2, S-3
(B) P-1, Q-4, R-3, S-2
(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-3, R-4, S-1

2023

**131.** The "purification fold" metric evaluates:
(A) The ratio of specific activity levels.
(B) The volume of enzyme recovered.
(C) Total enzymatic activity retained.
(D) Physical aggregation state of the protein.

2006

**132.** During continuous spectrophotometric assays, the reaction rate is determined by measuring:
(A) The final measured mass of product.
(B) Radioactivity of the final products.
(C) The specific isoelectric point shift.
(D) Absorbance change per unit time.

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2021

**133.** One International Unit (IU) of enzyme activity corresponds to the conversion of:
(A) 1 micromole of substrate per minute.
(B) 1 mole of substrate per second.
(C) 1 millimole of substrate per minute.
(D) 1 nanomole of substrate per hour.

2006

**134.** Which chemical is used for osmotic shock disruption?
(A) Strong solutions of trichloroacetic acid.
(B) High concentrations of SDS detergent.
(C) Absolute ethanol or other pure solvents.
(D) Sucrose buffer followed by distilled water.

2013

**135.** Consider the following statements regarding affinity chromatography:
I. It requires a specific ligand covalently attached to a matrix.
II. The target enzyme is eluted by adding free ligand to the buffer.
III. It strictly separates proteins by molecular weight.
IV. It can yield high purification folds in a single step.
V. It always utilizes an ion-exchange mechanism for binding.
Which of the above statements are correct?
(A) I, II and IV
(B) I, III and V
(C) II, IV and V
(D) I, II, III and IV

2001

**136.** Native-PAGE is chosen over SDS-PAGE primarily to:
(A) Preserve complexes and activity.
(B) Determine precise subunit molecular weight.
(C) Ensure all proteins carry a negative charge.
(D) Force proteins to separate by chain length.

2018

**137.** Which plot yields a direct determination of the Hill coefficient ($n_H$)?
(A) $v$ versus $[S]$
(B) $\log\left(\frac{v}{V_{\max}-v}\right)$ versus $\log[S]$
(C) $\frac{1}{v}$ versus $\frac{1}{[S]}$
(D) $\frac{[S]}{v}$ versus $[S]$

2024

**138.** Protein kinases utilize which molecule as the physiological phosphate donor?
(A) AMP
(B) ATP
(C) GTP
(D) Inorganic phosphate ($P_i$)

2014

**139.** Isoenzymes are accurately differentiated in the laboratory using:
(A) Electrophoretic mobility
(B) Ultracentrifugation
(C) Simple visible light microscopy
(D) Dialysis

NODIA

2009

**140.** Which observation supports the induced-fit model?
(A) Enzymes exhibit absolute specificity.
(B) Substrates fit into rigid pre-formed sites.
(C) Hexokinase closure upon glucose binding.
(D) Non-competitive inhibitors bind free enzyme.

2021

**141.** The initial velocity ($v_0$) of an enzyme-catalyzed reaction is defined as the slope of the curve when graphing:
(A) Substrate concentration versus velocity
(B) Enzyme concentration versus time
(C) $\frac{1}{v}$ versus $\frac{1}{[S]}$
(D) Product concentration versus time at $t \approx 0$

2019

**142.** A non-competitive inhibitor decreases the apparent $V_{\{}\max_{\}}$ because it:
(A) Competes effectively for active sites.
(B) Rapidly degrades the reaction substrate.
(C) Lowers functional enzyme concentration.
(D) Irreversibly precipitates enzymes.

2008

**143.** Which statement distinguishes competitive from uncompetitive inhibition?
(A) Competitive binds the ES complex; uncompetitive binds free E.
(B) Competitive can be overcome by high substrate; uncompetitive cannot.
(C) Competitive lowers $V_{\max}$; uncompetitive raises .$V_{\max}$
(D) Competitive alters the enzyme's primary structure; uncompetitive alters secondary structure.

2006

**144.** Which factor drives organic solvent precipitation of proteins?
(A) Increasing the solvent's dielectric constant.
(B) Direct modification of hydrophobic residues.
(C) Complete hydrolysis of polypeptide

backbones.
(D) Lowering the dielectric constant for attraction.

2018

**145.** Identify the correct general sequence for the multi-step purification of a typical intracellular enzyme:
(A) Homogenization, Salt Fractionation, Ion-Exchange Chromatography, Gel Filtration
(B) Gel Filtration, Homogenization, Ion-Exchange Chromatography, Salt Fractionation
(C) Ion-Exchange Chromatography, Salt Fractionation, Homogenization, Gel Filtration
(D) Salt Fractionation, Gel Filtration, Homogenization, Ion-Exchange Chromatography

2023

**146.** A critical application of zymography is the analysis of which specific class of enzymes?
(A) Transferases.
(B) Ligases.
(C) Proteases.
(D) Isomerases.

2010

**147.** Allosteric enzymes rarely follow Michaelis-Menten kinetics because their activity relies on:
(A) Cooperative binding of subunits.
(B) Irreversible covalent modifications.
(C) Absolute rigid structural domains.
(D) A strictly single-substrate mechanism.

2012

**148.** Which process exemplifies an irreversible regulatory mechanism?
(A) The phosphorylation of glycogen phosphorylase.
(B) Binding of ATP to aspartate transcarbamoylase.
(C) The allosteric feedback inhibition of kinase.
(D) Chymotrypsinogen cleavage to active enzyme.

2016

**149.** Which of the following is a mobile co-substrate that dissociates from the enzyme after the reaction is complete?
(A) FAD
(B) Biotin
(C) NAD+
(D) Heme

2017

**150.** Ribozymes challenge the historical definition of enzymes because they:
(A) Lack catalytic activity entirely.
(B) Function only at extreme temperatures.
(C) Are composed of RNA instead of amino acids.
(D) Require no cellular machinery for synthesis.

2018

**151.** Which term defines an organic, loosely bound cofactor that transports chemical groups between enzymes?
(A) Apoenzyme
(B) Prosthetic group
(C) Metalloenzyme
(D) Coenzyme

2007

**152.** Metal-activated enzymes differ from metalloenzymes in that they:
(A) Require environmental metal activation.
(B) Bind metal ions tightly with high affinity.
(C) Retain bound metals during purification.
(D) Exclusively use iron as the active metal.

2019

**153.** A ligase enzyme (EC 6) typically couples the formation of a new chemical bond with:
(A) The reduction of NAD+ molecules.
(B) The simple transfer of an electron.
(C) Hydrolysis of ATP phosphate bonds.
(D) Direct addition of water across bonds.

2013

**154.** What is the role of the amino acids that line the specificity pocket of an active site?
(A) To distinguish and bind correct structures.
(B) To directly transfer electrons to the substrate.
(C) To permanently lock the substrate in place.
(D) To undergo irreversible covalent cleavage.

2002

**155.** Consider the following statements regarding linear transformations of enzyme kinetics:
I. Lineweaver-Burk is statistically flawed due to overemphasizing data at low $[S]$.
II. Hanes-Woolf plot minimizes distortions caused by low $[S]$ errors.
III. Eadie-Hofstee plots $\frac{1}{v}$ against $[S]$.
IV. Dixon plot strictly uses variations in enzyme concentration.
Which of the above statements are correct?
(A) I and II
(B) III and IV

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(C) I, III and IV
(D) II, III and IV

2023

**156.** The energetic barrier that must be overcome for a reaction to proceed is quantified as:
(A) The total enthalpy change of reaction.
(B) The equilibrium constant of reaction.
(C) The free energy of activation ($\Delta G^{\dagger}$)
(D) The standard free energy of reaction ($\Delta G^{\circ}$).

2013

**157.** A general base catalyst speeds up a reaction by:
(A) Donating a proton to the substrate.
(B) Forming a tight covalent bond with substrate.
(C) Shielding a positive localized charge.
(D) Abstracting a proton for nucleophilicity.

2018

**158.** Mathematically, the turnover number ($k_{\{cat\}}$) is calculated by which equation?
(A) $k_{cat} = V_{\max} \times [E_t]$
(B) $k_{cat} = \frac{K_m}{V_{\max}}$
(C) $k_{cat} = \frac{V_{\max}}{[E_t]}$
(D) $k_{cat} = \frac{[E_t]}{V_{\max}}$

2003

**159.** In the Lineweaver-Burk plot, the x-intercept represents:
(A) $\frac{1}{V_{\max}}$
(B) $-\frac{1}{K_m}$
(C) $\frac{V_{\max}}{K_m}$
(D) $-K_m$

2015

**160.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option:
Assertion (A): Conformational selection asserts that substrates bind selectively to one of several pre-existing enzyme conformations.
Reason (R): Enzymes exist in a perfectly rigid state until the substrate forces a structural change.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2022

**161.** An ordered sequential mechanism can be distinguished from a random sequential mechanism by:
(A) Analyzing specific product inhibition patterns.
(B) Utilizing strictly single-substrate kinetic models.
(C) Measuring simply the standard free energy.
(D) Boiling the enzyme and measuring residual activity.

2024

**162.** How does an uncompetitive inhibitor alter the Michaelis-Menten parameters?
(A) It increases $K_m$ without changing .
(B) It decreases $V_{\max}$ without changing $K_m$. $V_{\max}$
(C) It decreases both $V_{\max}$ and apparent $K_m$ proportionately.
(D) It increases $V_{\max}$ while decreasing $K_m$.

2017

**163.** The primary target of the group-specific reagent iodoacetamide in enzyme mapping is usually:
(A) The sulfhydryl group of cysteine.
(B) The hydroxyl group of serine.
(C) The indole ring of tryptophan.
(D) The carboxyl group of aspartate.

2025

**164.** The optimal pH for an enzyme's activity is determined by:
(A) Random thermal motion of water molecules.
(B) Molecular weight of the enzyme protein.
(C) Concentration of the buffer in solution.
(D) The $pK_a$ of critical active site groups.

2007

**165.** The primary disadvantage of a discontinuous assay compared to a continuous assay is:
(A) It destroys the spectrophotometer cuvette.
(B) Difficulty in measuring rapid initial rates.
(C) It only works with radioactive labels.
(D) It never allows calculation of specific activity.

2007

**166.** A high specific activity indicates that:
(A) The enzyme is highly contaminated by proteins.
(B) The enzyme requires high salt concentration.
(C) Most of the protein mass is the active target enzyme.
(D) The enzyme has lost all regulatory properties.

2012

**167.** French press disruption is favored for bacterial cells because it:

(A) Chemically dissolves cell membrane parts.
(B) Selectively digests the peptidoglycan.
(C) Extracts purely lipid based components.
(D) Shears cells by rapid decompression.

2015

**168.** To precipitate a protein using an organic solvent without denaturing it, one must:
(A) Maintain temperature below zero.
(B) Control atmospheric pressure level.
(C) Control specific light exposure.
(D) Add heavy metal ions.

2012

**169.** Gel filtration chromatography excludes large proteins from its internal bead pores, meaning that:
(A) Large proteins elute first via void volume.
(B) Large proteins are retained longest to elute.
(C) Large proteins bind irreversibly to matrix.
(D) Large proteins are degraded by bead pores.

2016

**170.** Isoelectric focusing requires a stable:
(A) Temperature gradient
(B) Salinity gradient
(C) pH gradient
(D) Density gradient

2007

**171.** The defining characteristic of a homotropic allosteric modulator is that it:
(A) Is the normal substrate at regulatory sites.
(B) Is structurally unrelated to the substrate.
(C) Functions as a competitive inhibitor.
(D) Covalently modifies the enzyme structure.

2008

**172.** Protein phosphatase functions by:
(A) Adding a phosphate group via ATP.
(B) Cleaving the protein backbone chains.
(C) Removing phosphate by bond hydrolysis.
(D) Oxidizing specific tyrosine residues.

2012

**173.** Enzyme immobilization is primarily utilized in industrial processes to:
(A) Decrease the enzyme's thermal stability.
(B) Allow for easy enzyme recovery and reuse.
(C) Prevent the substrate from binding to the active site.
(D) Increase the water solubility of the catalyst.

2012

**174.** Which of the following accurately describes synzymes?
(A) Naturally occurring catalytic RNA molecules.
(B) Antibodies raised against transition states.
(C) Multienzyme complexes found in archaea.
(D) Artificially synthesized catalytic polymers.

2019

**175.** Match the chromatography types in List I with their corresponding elution methods in List II:
List I: (Chromatography)
P. Ion-exchange
Q. Affinity
R. Gel filtration
S. Hydrophobic interaction
List II: (Elution Method)
1. Decreasing salt gradient
2. Increasing salt gradient or pH change
3. Addition of competing free ligand
4. Isocratic elution (no gradient needed)
(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-4, R-2, S-3
(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-1, R-4, S-3

2025

**176.** Which type of enzyme relies on the presence of loosely bound $K^{+}$ or $Mg^{2+}$ ions from the medium for maximal activity?
(A) Metalloenzymes
(B) Ribozymes
(C) Abzymes
(D) Metal-activated enzymes

2018

**177.** Proteases belong to which major IUBMB enzyme class?
(A) Hydrolases (EC 3)
(B) Oxidoreductases (EC 1)
(C) Transferases (EC 2)
(D) Lyases (EC 4)

2008

**178.** The catalytic triad of chymotrypsin consists of which three amino acids?
(A) Glutamate, Cysteine, Lysine
(B) Asparagine, Histidine, Threonine
(C) Aspartate, Histidine, Serine
(D) Serine, Tyrosine, Arginine

2004

**179.** The transition state theory dictates that enzymes possess highest affinity for:
(A) Distorted reaction transition states.
(B) The substrate in its ground state.
(C) The product in its ground state.
(D) The un-bound allosteric modulator.

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2017

**180.** Binding energy in catalysis serves to:
(A) Increase activation energy barriers.
(B) Destabilize the enzyme's primary structure.
(C) Shift the equilibrium directly toward product.
(D) Offset the energy cost of transition states.

2018

**181.** Electrostatic catalysis accelerates reactions by:
(A) Forming transient covalent enzyme bonds.
(B) Stabilizing by charge distribution.
(C) Altering the global pH of the solution.
(D) Utilizing strong acid solutions.

2017

**182.** A competitive inhibitor affects the Michaelis-Menten plot by:
(A) Reducing the maximal height of the hyperbolic curve.
(B) Shifting the curve to the left, decreasing the apparent $K_m$.
(C) Shifting the curve to the right, increasing the apparent $K_m$.
(D) Transforming the curve into a straight line.

2014

**183.** Consider the following elements typically measured in a classic coupled enzyme assay:
I. Primary enzyme activity.
II. The rate of appearance/disappearance of the auxiliary reaction's measurable component.
III. The absolute mass of the primary enzyme.
Which of the above elements are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I

2006

**184.** In multi-substrate ping-pong mechanisms, Lineweaver-Burk plots at varying concentrations of the second substrate yield:
(A) Parallel lines.
(B) Lines intersecting on the y-axis.
(C) Lines intersecting on the negative x-axis.
(D) A single overlapping line.

2002

**185.** Consider the following statements concerning energetic principles of catalysis:
I. Enzymes do not alter the overall $\Delta G^{\circ}$ of a reaction.
II. Enzymes increase forward and reverse reaction rates equally.
III. Enzymes shift the final thermodynamic equilibrium position.
Which of the above statements are correct?
(A) I and III
(B) II and III
(C) I and II
(D) I

2025

**186.** Which molecule acts as a suicide substrate for the enzyme thymidylate synthase?
(A) Diisopropyl fluorophosphate (DIPF)
(B) 5-Fluorouracil
(C) Iodoacetamide
(D) Penicillin

2024

**187.** Increasing ionic strength up to a certain point stabilizes protein structure and solubility. This phenomenon is termed:
(A) Salting-in
(B) Salting-out
(C) Isoelectric precipitation
(D) Dialysis

2023

**188.** In an enzyme assay, the blank (or control) sample lacks:
(A) The reaction buffer system.
(B) The necessary cofactor ions.
(C) The enzyme or substrate.
(D) The spectrophotometer cuvette.

2009

**189.** A purification protocol reveals the specific activity rose from 10 U/mg to 100 U/mg. The purification fold is:
(A) 10
(B) 90
(C) 100
(D) 1000

2021

**190.** Lysozyme facilitates cell disruption by acting as a:
(A) Physical shear force generator.
(B) Chemical detergent solubilization agent.
(C) Catalyst hydrolyzing peptidoglycan.
(D) Chaotropic protein denaturing agent.

2001

**191.** Organic solvents precipitate proteins by decreasing the dielectric constant of the solution, which enhances:
(A) Hydrogen bonding with the water solvent.
(B) Attraction between charged proteins.
(C) Covalent disulfide bond formation.

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(D) Solubility of highly polar residues.

2007

**192.** Ion-exchange chromatography separates proteins on the basis of their:
(A) Overall molecular mass.
(B) Surface hydrophobicity.
(C) Specific affinity for an immobilized substrate.
(D) Net surface charge at a specific pH.

2014

**193.** Native-PAGE differs from SDS-PAGE in that it omits:
(A) SDS and reducing agents.
(B) Polyacrylamide gel matrices.
(C) The electrical field application.
(D) Standard gel staining procedures.

2007

**194.** The sequential (KNF) model for allosteric regulation requires:
(A) Binding stabilizes T-state of all others.
(B) Entire enzymes exist in T or R states.
(C) Propagating sequential changes.
(D) Cooperativity is physically impossible.

2016

**195.** Identify the correct practical order for the following steps of determining specific activity:
I. Measure total protein concentration (e.g., Bradford assay).
II. Calculate the ratio of total activity to total protein.
III. Perform an enzymatic assay to determine total activity.
IV. Obtain the purified enzyme sample.
(A) IV, III, I, II
(B) IV, I, III, II
(C) III, I, IV, II
(D) I, III, II, IV

2012

**196.** Zymogens provide a biological advantage because they:
(A) Eliminate the need for any substrate.
(B) Accelerate the catalytic turnover rate.
(C) Bypass the requirement for optimal pH.
(D) Prevent premature enzyme activity.

2017

**197.** An enzyme with a Km much lower than the physiological substrate concentration will follow:
(A) Zero-order kinetics.
(B) First-order kinetics.
(C) Second-order kinetics.
(D) Non-productive inhibition.

2003

**198.** Which classification describes the chemical nature of true enzymes?
(A) Inorganic catalysts.
(B) Simple strictly structural lipid parts.
(C) Complex proteinaceous or RNA molecules.
(D) Simple biological monosaccharides.

2019

**199.** A covalently bound, non-protein component required for an enzyme's activity is termed a/an:
(A) Apoenzyme
(B) Soluble coenzyme
(C) Reversible inhibitor
(D) Prosthetic group

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2012

**200.** Zinc coordinated within the active site of carbonic anhydrase identifies it as a:
(A) Metalloenzyme
(B) Metal-activated enzyme
(C) Ribozyme
(D) Pro-enzyme

2019

**201.** Which major IUBMB enzyme class catalyzes the movement of a chemical group from one molecule to another?
(A) Transferases (EC 2)
(B) Oxidoreductases (EC 1)
(C) Hydrolases (EC 3)
(D) Ligases (EC 6)

2012

**202.** The concept that substrates and active sites are structurally complementary primarily in the transition state refutes:
(A) The lock-and-key model.
(B) The fundamental tenets of kinetics.
(C) The induced-fit hypothesis.
(D) The principles of competitive inhibition.

2019

**203.** When using an enzyme, what is altered in the energy profile of a reaction?
(A) The activation energy.
(B) The energy of the reactants.
(C) The energy of the products.
(D) The total standard free energy difference.

2019

**204.** General acid catalysis is defined as catalysis driven by:
(A) The hydronium ion concentration.
(B) Full electron transfer redox.
(C) Proton donation from active site groups.
(D) Transient covalent linkage formation.

2012

**205.** Consider the following statements concerning the Michaelis constant ($K_m$):

I. It is exactly half of $V_{\max}$.
II. It is the substrate concentration at which velocity is half-maximal.
III. It represents an inverse measure of enzyme-substrate affinity.
IV. It changes when enzyme concentration is varied.

Which of the above statements are correct?

(A) I and IV
(B) II and III
(C) I, II and III
(D) II, III and IV

2024

**206.** In an enzyme active site, what does the y-intercept represent?

(A) $K_m$
(B) $\frac{V_{\max}}{K_m}$
(C) $-K_m$
(D) $V_{\max}$

2025

**207.** A random sequential multi-substrate mechanism is characterized by:

(A) Obligatory product release before binding.
(B) Either substrate can bind the enzyme first.
(C) Formation of a covalent enzyme intermediate.
(D) The complete absence of a ternary complex.

2025

**208.** Which inhibitory kinetic profile features lines intersecting on the x-axis of a Lineweaver-Burk plot?

(A) Pure competitive
(B) Uncompetitive
(C) Mixed (where $\alpha \neq \alpha$ â € ™)
(D) Pure non-competitive

2021

**209.** Diisopropyl fluorophosphate (DIPF) acts as an irreversible inhibitor by covalently modifying which residue?

(A) Serine
(B) Histidine
(C) Aspartate
(D) Cysteine

2001

**210.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option:
Assertion (A): Heavy metal ions generally act as irreversible inhibitors or strong denaturants.
Reason (R): Heavy metals disrupt structural disulfide bonds and coordinate with free sulfhydryl groups.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2022

**211.** Continuous spectrophotometric assays rely on:

(A) Monitoring real-time absorbance changes.
(B) Destroying the sample at interval points.
(C) Manual titration of the final product.
(D) Radioactive decay tracking of products.

2012

**212.** Percentage yield compares:

(A) Final specific activity to initial specific activity.
(B) Final total protein to initial total protein.
(C) Molecular weight of purified protein to the crude mixture.
(D) Total enzymatic activity remaining to initial total enzymatic activity.

2005

**213.** Osmotic shock is useful for isolating proteins from the:

(A) Inner mitochondrial matrix.
(B) Bacterial periplasmic space.
(C) Nuclear core complex.
(D) Endoplasmic reticulum lumen.

2001

**214.** The term "salting-out" describes the phenomenon where high salt concentrations lead to:

(A) Increased protein solubility.
(B) Denaturation of the primary sequence.
(C) Competition for water leading to aggregation.
(D) Selective precipitation of nucleic acids.

2001

**215.** Affinity chromatography relies on which characteristic of the target enzyme?

(A) Its bulk isoelectric point.
(B) Its surface hydrophobicity.
(C) Its precise overall molecular weight.
(D) Specific biological ligand interactions.

2012

**216.** SDS-PAGE ensures proteins separate by mass by:

(A) Applying a uniform negative charge per unit mass.

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(B) Eliminating all charge from the proteins.
(C) Retaining the native folded 3D shape.
(D) Cross-linking proteins to standard sizes.

2007

**217.** An allosteric enzyme graph plotting velocity versus substrate concentration yields a:
(A) Straight line
(B) Sigmoidal curve
(C) Hyperbola
(D) Parabola

2016

**218.** Regulatory cascades utilize kinase enzymes to exert control through:
(A) Irreversible peptide cleavage.
(B) Covalent phosphorylation.
(C) Spontaneous denaturation.
(D) Direct transcription blocking.

2018

**219.** Multienzyme complexes benefit from "substrate channeling," which means:
(A) Intermediates stay within the complex.
(B) Intermediates are released into the cytosol.
(C) The complex uses a single active site for all steps.
(D) Coenzymes are entirely unnecessary.

2001

**220.** Abzymes merge concepts of enzymology and immunology by utilizing:
(A) T-cell receptors that hydrolyze peptides.
(B) RNA molecules that act as antibodies.
(C) Antibodies that bind transition states.
(D) Synzymes that mimic immune cells.

2021

**221.** Which of the following terms describes the complete, catalytically active form of an enzyme including its non-protein components?
(A) Apoenzyme
(B) Zymogen
(C) Holoenzyme
(D) Coenzyme

2021

**222.** An enzyme containing an essential, tightly bound $Zn^{2+}$ ion that is an integral part of its structure is termed a:
(A) Pro-enzyme
(B) Metal-activated enzyme
(C) Metalloenzyme
(D) Zymogen

2017

**223.** EC Class 4 designates Lyases, which catalyze:
(A) Hydrolytic cleavage of bonds.
(B) Cleavage by elimination leaving double bonds.
(C) Joining two molecules using ATP.
(D) The transfer of electrons.

2015

**224.** The active site occupies a small volume of the total enzyme. The rest of the protein serves to:
(A) Increase weight to avoid renal clearance.
(B) Act as a large non-specific substrate sink.
(C) Generate purely metabolic heat.
(D) Maintain the 3D architecture of the active site.

2007

**225.** Match the kinetic parameters in List I with their physical meanings in List II:
List I: (Kinetic Parameters)
P. $V_{\max}$
Q. $K_m$
R. $k_{\{cat\}}$
S. $\frac{k_{cat}}{K_m}$
List II: (Physical Meanings)
1. Maximum catalytic rate at saturation
2. Measure of catalytic efficiency
3. Turnover number per active site
4. Substrate concentration at half $V_{\max}$
(A) P-1, Q-4, R-3, S-2
(B) P-3, Q-2, R-1, S-4
(C) P-1, Q-4, R-2, S-3
(D) P-4, Q-1, R-3, S-2

2023

**226.** According to the induced-fit model, initial binding of the substrate forces:
(A) Instantaneous destruction of the substrate.
(B) Global denaturation of the enzyme.
(C) A structural shift aligning catalytic groups.
(D) Rigid binding without enzyme movement.

2013

**227.** A reaction reaches the transition state when:
(A) Molecules are at the peak of the energy barrier.
(B) Standard free energy is minimized.
(C) The equilibrium constant is exactly zero.
(D) The substrate matches the product structure.

2004

**228.** Metal-ion catalysis can facilitate reactions by doing all of the following EXCEPT:
(A) Shielding negative substrate charges.
(B) Mediating oxidation-reduction events.
(C) Promoting attacks via water polarization.
(D) Irreversibly denaturing the protein backbone.

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2022

**229.** The steady-state assumption assumes that:
(A) The substrate concentration never drops.
(B) ES complex concentration remains constant.
(C) The reaction velocity equals zero.
(D) The product reversibly forms substrate.

2013

**230.** In a linear Lineweaver-Burk plot, the slope equals:
(A) $\frac{1}{V_{max}}$
(B) $\frac{V_{max}}{K_m}$
(C) $\frac{K_m}{V_{max}}$
(D) $-\frac{1}{K_m}$

2009

**231.** An ordered sequential bi-bi mechanism means:
(A) Substrates bind in a random fashion.
(B) First product releases before second substrate binds.
(C) Enzyme relies on purely covalent catalysis.
(D) First substrate must bind before second substrate binds.

2022

**232.** An uncompetitive inhibitor impacts the Lineweaver-Burk plot by creating:
(A) Parallel lines with shifting intercepts.
(B) Intersecting lines at the y-axis.
(C) Intersecting lines on the x-axis.
(D) Overlapping straight lines.

2008

**233.** Transition-state analogs act as potent inhibitors because they:
(A) Are bulky and block entry to the site.
(B) Chemically destroy the active site.
(C) Rapidly precipitate the enzyme.
(D) Exploit binding energy intended for the transition state.

2011

**234.** The Q10 temperature coefficient dictates the factor by which the enzymatic rate changes following a:
(A) 1 degree Celsius increase.
(B) 10 degree Celsius increase.
(C) 100 degree Celsius increase.
(D) 10-fold increase in pressure.

2015

**235.** Consider the following statements regarding discontinuous assays:
I. They involve stopping the reaction at specific times.
II. They are optimal for extremely fast reactions with spectral changes.
III. They require a separate analytical step to measure products.
IV. They provide continuous real-time kinetic curves.
V. They utilize quenching agents like TCA or heat.
Which of the above statements are correct?
(A) I, III and V
(B) II, IV and V
(C) I, II and III
(D) I, III, IV and V

2017

**236.** The term "specific activity" quantifies:
(A) Total number of enzyme molecules.
(B) Overall volumetric yield.
(C) Enzyme activity per unit of total protein.
(D) Physical molecular weight of the enzyme.

2002

**237.** Which physical disruption method utilizes high-frequency sound waves?
(A) French press
(B) Bead beating
(C) Osmotic shock
(D) Sonication

2013

**238.** Isoelectric precipitation relies on minimizing solubility by adjusting pH to the point where:
(A) The protein attains maximum positive charge.
(B) The protein attains maximum negative charge.
(C) The protein net charge is zero.
(D) The protein molecule unfolds.

2002

**239.** Gel filtration separates proteins on the basis of:
(A) Specific biological affinity interaction.
(B) Molecular size and hydrodynamic shape.
(C) Hydrophobic surface patches.
(D) Isoelectric point measurements.

2024

**240.** The advantage of running 2D electrophoresis (IEF followed by SDS-PAGE) is the ability to resolve proteins by both:
(A) Hydrophobicity and specific activity.
(B) Subunit number and affinity.
(C) A-helices and β-sheets.
(D) Isoelectric point and molecular mass.

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2004

**241.** A Hill coefficient ($n_H$) exceeding 1.0 diagnoses:
(A) Positive cooperative ligand binding.
(B) Negative cooperativity.
(C) Non-cooperative kinetics.
(D) Zero-order kinetics.

2007

**242.** Protein cascades ensure regulation through:
(A) Rapid spontaneous degradation.
(B) Eliminating the need for specific substrates.
(C) Exponential signal amplification.
(D) Avoiding cellular ATP usage.

2006

**243.** The biological importance of isoenzymes like LDH lies in their ability to:
(A) Adapt reactions to tissue requirements.
(B) Bypass thermodynamic laws.
(C) Utilize different coenzymes in every tissue.
(D) Avoid genetic transcription.

2019

**244.** Ribozymes prove that:
(A) Proteins do not exist in life.
(B) DNA directly catalyzes metabolic steps.
(C) Lipids form specific active site pockets.
(D) RNA forms complex 3D structures for catalysis.

2018

**245.** A covalently bound, non-protein ring structure (like heme) is defined as a:
(A) Loosely bound coenzyme.
(B) Reversible competitive inhibitor.
(C) Purely structural domain.
(D) Prosthetic group.

2014

**246.** Metalloenzymes differ from metal-activated enzymes due to their reliance on:
(A) Loosely exchangeable alkali metal ions.
(B) Tightly bound metal ions in the active site.
(C) Organic non-metallic cofactors.
(D) Metal salts added post-purification.

2004

**247.** An enzyme that directly transfers a functional group is categorized as a:
(A) Hydrolase.
(B) Lyase.
(C) Ligase.
(D) Transferase.

2024

**248.** The active site microenvironment relies on specific amino acid side chains to:
(A) Position and stabilize transition states.
(B) Maximize steric hindrance.
(C) Permanently bind substrates.
(D) Increase local thermodynamic temperature.

2025

**249.** Conformational selection argues that the enzyme:
(A) Forms an absolute rigid template.
(B) Binds only the transition state.
(C) Alters the overall $\Delta G^{\circ}$.
(D) Samples multiple states prior to binding.

2016

**250.** The energetic quantity lowered by enzymatic catalysis is the:
(A) Free energy of activation ($\Delta G^{\dagger}$)
(B) Standard enthalpy of reaction
(C) Standard free energy of reaction ($\Delta G^{\circ}$)
(D) Entropy of the system

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**********

# ANSWERS

**1.** (B) A catalytic RNA molecule
Ribozymes are specialized RNA molecules possessing catalytic activity, challenging the traditional view that all enzymes are proteins. They facilitate essential biological processes, such as peptide bond formation in ribosomes and RNA splicing. By folding into complex three-dimensional structures, they create active sites that stabilize transition states and accelerate specific biochemical reactions within cells.

**2.** (A) An apoenzyme with its cofactor
A holoenzyme represents the complete, catalytically active form of an enzyme, consisting of the protein component and any necessary non-protein factors. The protein part alone is known as an apoenzyme, which remains inactive until it binds with specific cofactors, such as metal ions or organic coenzymes, to form the functional biological unit required for catalysis.

**3.** (C) Hold tightly bound essential metal ions
Metalloenzymes are characterized by the presence of tightly bound metal ions that are integral to the enzyme structure and catalytic mechanism. These ions remain associated with the protein throughout purification. In contrast, metal-activated enzymes require loosely associated metal ions from the surrounding medium to enhance activity, often losing their function if the metal is removed.

**4.** (D) Hydrolases
Enzymes classified under the third major class of the International Union of Biochemistry and Molecular Biology are known as hydrolases. These catalysts facilitate the cleavage of chemical bonds, such as peptide, ester, or glycosidic bonds, through the addition of water. They play critical roles in digestion and the breakdown of various cellular polymers into smaller subunits.

**5.** (C) It represents the bulk of total enzyme volume.
The active site is a relatively small, specialized cleft or pocket within the large enzyme structure where substrate binding and catalysis occur. While the bulk of the enzyme provides the necessary architectural support to maintain the precise three-dimensional arrangement of the active site, the actual catalytic region constitutes only a minor fraction of the total molecular volume.

**6.** (B) Binding induces an enzyme conformational change.
The induced-fit model suggests that enzymes are flexible structures rather than rigid templates. Upon the initial interaction with a substrate, the enzyme undergoes a conformational change that reshapes the active site to achieve a more precise fit. This dynamic adjustment optimizes the orientation of catalytic functional groups, facilitating the stabilization of the transition state during the reaction.

**7.** (D) Lowering activation energy to stabilize transition states
Binding energy is the free energy released during the formation of multiple weak interactions between an enzyme and its substrate. This energy is primarily utilized to lower the activation energy of the reaction. By providing a medium that specifically stabilizes the high-energy transition state relative to the ground state, enzymes significantly accelerate the overall rate of chemical transformations.

**8.** (A) Electrostatic catalysis uses hydrophobic bonds.
Electrostatic catalysis primarily involves the use of charged groups within the enzyme active site to stabilize transition states or redistribute electronic density. It relies on ionic interactions and dipoles rather than hydrophobic bonds. Hydrophobic interactions generally contribute to substrate binding and the exclusion of water from the active site, whereas electrostatic effects specifically influence the energetic stability of polar intermediates.

**9.** (C) $k_{cat}$ - Turnover number of the enzyme
The turnover number, denoted as $k_{cat}$, represents the maximum number of substrate molecules converted to product by a single active site per unit of time when the enzyme is fully saturated. It is a fundamental measure of an enzyme's catalytic rate. Other parameters like $K_m$ reflect

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substrate affinity, while the ratio of $k_{\{cat\}}$ to $K_m$ indicates overall catalytic efficiency.

**10.** (A) Both A and R are true and R is the correct explanation of A.
The Lineweaver-Burk plot utilizes double reciprocal values, which causes small experimental errors at low substrate concentrations to be disproportionately magnified. Since low concentrations result in large reciprocal values, any minor deviation significantly shifts the data points on the graph. Consequently, this linear transformation can lead to less accurate determinations of kinetic constants compared to other modern statistical methods.

**11.** (D) Product release occurs before second substrate binding.
In a Ping-Pong or double-displacement mechanism, the enzyme reacts with the first substrate to release a product and form a modified enzyme intermediate. Only after the first product is released does the second substrate bind to the modified enzyme to complete the reaction. This mechanism is characteristic of many aminotransferases and does not involve a ternary complex formation.

**12.** (B) Uncompetitive inhibition
Uncompetitive inhibition occurs when an inhibitor binds exclusively to the enzyme-substrate complex rather than the free enzyme. This binding typically takes place at a site created only after the substrate has bound. This interaction reduces both the maximum velocity and the apparent Michaelis constant, resulting in parallel lines on a double-reciprocal plot during kinetic analysis of the enzyme.

**13.** (C) Suicide substrates
Suicide substrates, also known as mechanism-based inactivators, are relatively unreactive molecules that the enzyme initially processes as a normal substrate. During the catalytic cycle, the enzyme's own mechanism converts the inhibitor into a highly reactive intermediate. This latent functional group then forms a permanent covalent bond with a residue in the active site, leading to irreversible enzyme inactivation.

**14.** (D) The reaction rate increase factor per 10°C rise.
The temperature coefficient, or $Q_{10}$, is a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by ten degrees Celsius. For most enzymatic reactions, the $Q_{10}$ value is approximately two, meaning the reaction rate doubles with every ten-degree rise. This factor helps in understanding the thermal sensitivity of metabolic processes.

**15.** (A) I and III
In a coupled enzyme assay, the primary reaction produces a product that is immediately consumed by a second, auxiliary enzyme to produce a measurable signal. To ensure accurate measurement, the primary reaction must be the rate-limiting step, and the secondary reaction must be fast enough to prevent product accumulation. This allows researchers to monitor reactions that do not produce detectable changes.

**16.** (B) Total protein drops while target activity is retained.
Specific activity is defined as the total units of enzyme activity divided by the total mass of protein in a sample. During a successful purification process, unwanted proteins are removed, leading to a significant decrease in total protein content. If the target enzyme's activity is preserved, the ratio of activity to protein increases, indicating a higher degree of purity.

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**17.** (B) Risk of protein denaturation by chemicals.
Chemical methods of cell disruption use detergents, solvents, or alkalis to permeabilize the cell membrane and release intracellular contents. While these methods are often simpler than physical techniques, the chemicals used can interact with the target proteins. This carries a significant risk of altering the protein's native structure or causing complete denaturation, potentially destroying the enzyme's catalytic activity.

**18.** (A) Competing for protein hydration water.
Salting-out occurs when high concentrations of neutral salts, such as ammonium sulfate, are added to a protein solution. The salt ions become highly hydrated, effectively competing with the protein molecules for the available water. As the hydration shell around the proteins is stripped away, hydrophobic patches on the protein surfaces interact, leading to aggregation and precipitation from the solution.

**19.** (C) Q, R, P
Size-exclusion chromatography, or gel filtration, separates proteins based on their molecular size. Large molecules are excluded from the internal pores of the stationary phase beads and travel through the void volume, eluting first. Smaller molecules enter the pores and take a longer path, eluting later. Therefore, the heaviest protein (120 kDa) elutes first, followed by 60 kDa and 15 kDa.

**20.** (D) Specific enzyme activity within a gel matrix.
Zymography is an electrophoretic technique used to detect enzymatic activity, particularly proteases, within a polyacrylamide gel. A substrate is copolymerized with the gel matrix during preparation. After electrophoresis and incubation in an appropriate buffer, the active enzymes digest the substrate. Subsequent staining reveals clear zones where the substrate was degraded, allowing the visualization of active enzyme bands.

**21.** (B) Hill coefficient
Allosteric enzymes display sigmoidal kinetics due to cooperative interactions between subunits. The Hill coefficient is a mathematical parameter used to quantify the degree of this cooperativity. A coefficient greater than one indicates positive cooperativity, where ligand binding at one site increases affinity at others, while a coefficient equal to one suggests independent binding following traditional Michaelis-Menten kinetics.

**22.** (C) Irreversible cleavage of a precursor.
Zymogens are inactive enzyme precursors that require structural modification to become functional. The activation of a zymogen typically involves the irreversible cleavage of one or more specific peptide bonds by a protease. This proteolytic processing removes an inhibitory segment of the protein, allowing the molecule to fold into its catalytically active conformation, ensuring enzyme activity occurs only when needed.

**23.** (D) Sequence and physical properties.
Isoenzymes are multiple forms of an enzyme that catalyze the same chemical reaction but are characterized by different amino acid sequences. These differences arise from distinct genetic loci or alternative splicing. Consequently, isoenzymes often exhibit unique physical and chemical properties, such as different electrophoretic mobilities, kinetic parameters, and regulatory behaviors, allowing them to function efficiently in specific tissues or conditions.

**24.** (A) Substrate concentration is much higher than $K_m$
Zero-order kinetics occur when the rate of an enzymatic reaction is independent of the substrate concentration. This condition is met when the substrate concentration is much higher than the Michaelis constant, leading to the saturation of all available enzyme active sites. At this point, the reaction proceeds at its maximum velocity, and further increases in substrate do not enhance the rate.

**25.** (C) II and IV
The structural organization of enzymes often involves non-protein components. Prosthetic groups are organic molecules that are tightly or covalently bound to the protein. Holoenzymes represent the complete, catalytically active complex consisting of the protein part and its necessary cofactors. In contrast, apoenzymes are the protein portions alone and remain inactive until the required cofactors are associated with them.

**26.** (B) Tightly bound FAD prosthetic groups.
Flavin adenine dinucleotide (FAD) serves as a prosthetic group for many oxidoreductases. Unlike soluble coenzymes that dissociate after the reaction, FAD is tightly or covalently bound to the enzyme structure. This permanent association allows FAD to participate in electron transfer reactions within the protein framework, facilitating the temporary storage and movement of electrons during the catalytic cycle.

**27.** (A) Lower transition state free energy.
Metal ions facilitate enzymatic catalysis through several mechanisms, including the stabilization of negative charges and the orientation of substrates. By coordinating with the substrate or the active site residues, these ions help stabilize the high-energy transition state. This reduction in transition state free energy lowers the activation energy barrier, thereby accelerating the overall rate of the chemical reaction.

**28.** (C) Lyases (EC 4) cleave bonds strictly by hydrolysis.
The classification of enzymes is based on the type of reaction they catalyze. Lyases, belonging

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to class EC 4, are responsible for the cleavage of various chemical bonds by means other than hydrolysis or oxidation. This often results in the formation of double bonds or the addition of groups to double bonds. Hydrolysis is specifically the domain of the EC 3 class.

**29.** (D) Hydrogen bond - 2.5 to 3.5 Angstroms
Interactions within the active site occur at specific molecular distances. Hydrogen bonds, which are critical for substrate binding and transition state stabilization, typically form between atoms spaced 2.5 to 3.5 Angstroms apart. Van der Waals forces operate at shorter ranges, while salt bridges involve ionic interactions. Covalent bonds are much shorter, usually measuring around 1 to 1.5 Angstroms in length.

**30.** (A) P-1, Q-2, R-3, S-4
Reversible inhibitors are distinguished by their specific effects on kinetic parameters. Competitive inhibitors increase the Michaelis constant but leave maximum velocity unchanged. Non-competitive inhibitors decrease maximum velocity while the Michaelis constant remains constant. Uncompetitive inhibitors cause a decrease in both parameters. Mixed inhibitors typically increase the Michaelis constant and decrease the maximum velocity, reflecting interactions with both the enzyme and the complex.

**31.** (B) Conformational Selection model.
The conformational selection model proposes that enzymes naturally exist in a dynamic equilibrium among multiple structural states. A substrate selectively binds to the specific conformation that is most complementary to its shape, shifting the equilibrium toward that state. This differs from the induced-fit model, where the substrate forces the enzyme to change shape only after the initial binding event occurs.

**32.** (D) Energy required to reach the transition state.
Activation energy is the minimum amount of energy that must be provided to chemical reactants to trigger a particular reaction. In the context of catalysis, it represents the energy barrier between the ground state of the reactants and the high-energy transition state. Enzymes function by providing an alternative pathway that lowers this energy requirement, thereby increasing the reaction rate.

**33.** (A) Covalent catalysis
Covalent catalysis involves the formation of a transient, high-energy covalent bond between the enzyme and the substrate during the reaction. In chymotrypsin, a specific serine residue in the active site acts as a nucleophile to attack the substrate, forming an acyl-enzyme intermediate. This intermediate is subsequently hydrolyzed to release the product, effectively accelerating the reaction by providing a multi-step pathway.

**34.** (C) ES formation rate equals its breakdown rate.
The steady-state assumption is a fundamental principle used in deriving the Michaelis-Menten equation. It posits that after a very brief initial period, the concentration of the enzyme-substrate complex remains constant over time. This implies that the rate of formation of the complex from free enzyme and substrate is exactly balanced by its rate of breakdown into products or back into reactants.

**35.** (B) I, II and V
The Eadie-Hofstee plot is a linear transformation of the Michaelis-Menten equation that graphs velocity against the ratio of velocity to substrate concentration. This plotting method is advantageous because it provides a more uniform distribution of experimental errors across the substrate range. Unlike the Lineweaver-Burk plot, it does not disproportionately weight data collected at very low substrate concentrations, improving statistical reliability.

**36.** (D) Substrates bind in a specific sequence.
Ordered sequential kinetics describe a multi-substrate reaction where the substrates must bind to the enzyme in a mandatory, specific order before catalysis can proceed. Similarly, the resulting products are released in a defined sequence. This mechanism involves the formation of a ternary complex where all substrates are bound simultaneously to the enzyme active site before any chemical transformation occurs.

**37.** (B) It directly calculates the inhibition constant ($K_i$).
The Dixon plot is a graphical method used in enzyme kinetics to determine the inhibition constant, $K_i$. By plotting the reciprocal of the reaction velocity against the inhibitor concentration at multiple fixed substrate concentrations, the resulting lines intersect at a point that corresponds to the value of $K_i$. This

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method is particularly useful for identifying and quantifying the potency of competitive and non-competitive inhibitors.

**38.** (C) Irreversible group-specific reagent.
Diisopropyl fluorophosphate (DIPF) is a classic example of an irreversible group-specific reagent. It specifically targets and covalently modifies the hydroxyl group of serine residues within the active sites of serine proteases and acetylcholinesterase. Because the resulting covalent bond is stable and permanent, the enzyme is completely and irreversibly inactivated, making DIPF a highly potent toxin and a valuable tool for mapping active sites.

**39.** (D) High ionic strength increases solubility.
While low concentrations of salt can increase protein solubility, a phenomenon known as salting-in, very high ionic strength typically leads to protein precipitation, or salting-out. This occurs because the high concentration of ions competes for the water molecules needed to hydrate the protein. In contrast, optimal pH and temperature are essential for maintaining the native structure and ionization states required for catalysis.

**40.** (A) Monitors reaction progress over time.
A continuous enzyme assay allows for the real-time monitoring of reaction progress, typically by measuring changes in light absorbance or fluorescence as the reaction occurs. This provides a direct visualization of the initial velocity and ensures that the rate remains linear during the measurement period. Discontinuous assays, conversely, involve stopping the reaction at fixed intervals to measure the total product formed.

**41.** (B) 1 mole of substrate per second
The Katal is the SI unit of catalytic activity used to quantify the power of an enzyme. It is defined as the amount of enzyme that catalyzes the transformation of one mole of substrate per second under specified reaction conditions. Because this unit is very large, enzyme activity in laboratory settings is more frequently expressed in smaller units, such as the International Unit.

**42.** (B) Acoustic cavitation shear stress.
Sonication is a physical method of cell disruption that utilizes high-frequency sound waves to create microscopic bubbles in a liquid medium. The rapid formation and violent collapse of these bubbles, a process known as acoustic cavitation, generate intense localized shear stress and pressure changes. These forces effectively tear open cell membranes and walls, releasing the intracellular components into the surrounding buffer.

**43.** (A) Minimum solubility at the isoelectric point.
The isoelectric point is the specific pH at which a protein molecule carries no net electrical charge. At this pH, the lack of electrostatic repulsion between protein molecules allows them to aggregate through hydrophobic interactions and Van der Waals forces. Consequently, proteins exhibit their minimum solubility at their isoelectric point, making this a useful principle for selective precipitation during purification.

**44.** (C) Carry a negative charge and bind matrix.
When the surrounding pH is higher than the isoelectric point of a protein, the protein carries a net negative charge. At pH 8.0, an enzyme with a pI of 5.0 will be negatively charged and will interact strongly with a positively charged anion-exchange matrix, such as DEAE-cellulose. This selective binding allows the protein to be retained on the chromatography column while others elute.

**45.** (A) III, IV, II, I
Purifying an intracellular enzyme typically follows a logical sequence starting with cell disruption to release the protein. This is followed by centrifugation to remove insoluble cell debris and organelles. Bulk purification steps, such as ammonium sulfate precipitation, are then used to concentrate the protein. Finally, highly selective techniques like affinity chromatography are employed to isolate the target enzyme in its purest form.

**46.** (C) Charge, size, and 3D shape
Native-PAGE is an electrophoretic technique that separates proteins in their folded, functional state. Unlike SDS-PAGE, which denatures proteins and separates them based on mass alone, native-PAGE separation is influenced by the protein's net charge, its overall size, and its specific three-dimensional shape. This method is particularly useful for studying protein-protein interactions, oligomeric states, and maintaining enzymatic activity after separation.

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**47.** (B) All subunits share the same conformation.
The concerted model, or MWC model, of allosteric regulation posits that all subunits of a multimeric enzyme must exist in the same conformational state, either the low-affinity T state or the high-affinity R state. The binding of a ligand shifts the equilibrium between these two global states. This model implies that the transition occurs simultaneously across all subunits, maintaining the enzyme's internal symmetry.

**48.** (D) Serine, Threonine, Tyrosine
Phosphorylation is a major reversible covalent modification used to regulate enzyme activity. This process involves the transfer of a phosphate group from ATP to the hydroxyl group of specific amino acid side chains. Serine, threonine, and tyrosine are the primary targets because they possess the necessary nucleophilic hydroxyl groups. The addition of the negatively charged phosphate group induces conformational changes that activate or inhibit the enzyme.

**49.** (A) Regulate tissue metabolic requirements.
The existence of multiple isoenzymes for lactate dehydrogenase allows different tissues to tailor their metabolic pathways according to specific physiological needs. For instance, heart tissue requires isoenzymes that favor aerobic metabolism, while skeletal muscle utilizes forms better suited for anaerobic glycolysis. By expressing different subunit combinations, the body ensures that the catalytic properties of the enzyme match the metabolic demands of each organ.

**50.** (B) The reaction's transition-state analog
Abzymes, or catalytic antibodies, are engineered by raising an immune response against a stable transition-state analog. Because these antibodies are designed to bind the high-energy intermediate of a reaction more tightly than the substrate itself, they stabilize the transition state in a manner similar to natural enzymes. This stabilization lowers the activation energy, enabling the antibody to catalyze specific chemical transformations.

**51.** (A) Apoenzymes require coenzymes for activity.
An apoenzyme is the purely protein-based portion of an enzyme, which lacks the non-protein factors necessary for its biological function. To become catalytically active, the apoenzyme must associate with a coenzyme, which is a small organic molecule that often participates directly in the chemical reaction. The combination of the apoenzyme and the required coenzyme or metal ion constitutes the functional holoenzyme.

**52.** (D) Free $Mg^{2+}$ requirement for kinases.
Metal-activated enzymes are distinguished by their requirement for loosely associated metal ions from the surrounding solution to achieve maximal catalytic activity. Kinases, for example, typically require free magnesium ions to coordinate with ATP, facilitating the transfer of phosphate groups. Unlike metalloenzymes, where the metal is a permanent structural component, these ions can often be exchanged or removed without permanently denaturing the protein.

**53.** (C) Isomerase
Isomerases are a class of enzymes that catalyze the interconversion of isomers, involving the rearrangement of atoms within a single molecule. This includes reactions such as epimerization, which changes the configuration around a specific chiral center, or the conversion between aldose and ketose sugars. These enzymes facilitate structural or geometric changes without altering the overall chemical formula of the substrate.

**54.** (C) It prevents conformational shifts.
The active site of an enzyme is a specialized cleft designed to facilitate catalysis through a microenvironment that promotes substrate binding via weak, non-covalent interactions. Far from being rigid, the active site often undergoes significant conformational shifts, such as induced fit, to optimize the orientation of catalytic groups. These dynamic movements are essential for stabilizing the transition state and promoting the efficient conversion of substrate to product.

**55.** (B) I, III and IV
Several models describe enzyme-substrate interactions. The lock-and-key model is outdated because it fails to explain how enzymes stabilize high-energy transition states. Conformational selection posits that enzymes sample multiple shapes before binding, rather than being forced into shape by the substrate. Furthermore, active sites are evolved to be complementary to the transition state, not the ground state, which is essential for lowering the reaction's activation energy.

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**56.** (A) Decreasing the reaction energy barrier.
Transition state stabilization is the primary mechanism by which enzymes accelerate chemical reactions. By binding the high-energy intermediate more tightly than either the substrate or the product, the enzyme provides a pathway with a lower activation energy. This reduction in the energy barrier allows a greater proportion of reactant molecules to reach the transition state at a given temperature, significantly increasing the reaction rate.

**57.** (B) Covalent catalysis
Covalent catalysis involves the formation of a temporary covalent bond between the enzyme and the substrate. This occurs when a nucleophilic functional group on an amino acid side chain, such as the hydroxyl group of serine or the sulfhydryl group of cysteine, attacks the substrate. This process creates a transient intermediate that provides a more energetically favorable pathway for the reaction, effectively lowering the activation energy barrier.

**58.** (A) $[S] = K_m$
The Michaelis constant, $K_m$, is defined as the substrate concentration at which the reaction velocity is exactly half of the maximum velocity. This relationship is a fundamental characteristic of the Michaelis-Menten equation. Mathematically, when the substrate concentration equals $K_m$, the denominator of the equation becomes twice $K_m$ , resulting in a velocity that is one-half of the $V_{\{\max\}}$ value for that specific enzyme.

**59.** (A) $\frac{[S]}{v}$ vs $[S]$
The Hanes-Woolf plot is a linear transformation of the Michaelis-Menten equation that graphs the ratio of substrate concentration to velocity against the substrate concentration. This plotting method is often preferred over the Lineweaver-Burk plot because it provides a more accurate distribution of data points across the substrate range. The resulting straight line allows for the clear determination of both the maximum velocity and the Michaelis constant.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Transaminases typically follow a Ping-Pong bi-bi mechanism, characterized by the alternating binding of substrates and release of products. During the first half-reaction, the amino group from the first substrate is transferred to the enzyme's pyridoxal phosphate prosthetic group, releasing the first product. The second substrate then binds to the modified enzyme to receive the amino group, completing the catalytic cycle and regenerating the original enzyme.

**61.** (D) Both free enzyme and enzyme-substrate complex
Mixed inhibition is a type of reversible inhibition where the inhibitor can bind to both the free enzyme and the enzyme-substrate complex. The binding site for the inhibitor is typically distinct from the active site. This dual interaction results in a decrease in the maximum velocity and an alteration of the Michaelis constant, depending on the relative affinities of the inhibitor for the different enzyme forms.

**62.** (C) Target the active site by resembling the substrate.
Affinity labels are irreversible inhibitors specifically designed to mimic the structure of a natural substrate. This structural resemblance directs the molecule to the enzyme's active site. Once bound, a reactive functional group on the affinity label forms a permanent covalent bond with a nearby amino acid residue. This specificity distinguishes them from group-specific reagents, which react with specific functional groups anywhere on the protein surface.

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**63.** (B) Altering ionization of active site residues.
Enzyme activity is highly dependent on the pH of the surrounding medium, as it influences the ionization state of critical amino acid side chains within the active site. Shifts in pH away from the optimum can lead to the gain or loss of protons on catalytic residues, disrupting their ability to facilitate the reaction. Furthermore, extreme pH changes can destabilize the protein's three-dimensional structure, leading to denaturation.

**64.** (D) Allows measurement of silent products.
Coupled enzyme assays are invaluable when the product of the primary reaction cannot be directly measured by standard optical techniques. By introducing a secondary, auxiliary enzyme that uses this product to generate a change in absorbance or fluorescence, the progress of the otherwise "silent" primary reaction can be monitored. This method provides a convenient and sensitive way to quantify enzymatic activity for a wide range of biological processes.

**65.** (C) I and III
Enzyme purification is assessed using several key metrics. Specific activity is the ratio of total enzymatic activity to the total amount of protein, reflecting purity. Percentage yield indicates the proportion of the initial activity that remains after the purification steps. A successful purification should show an increase in specific activity and a high percentage yield, while the purification fold represents the factor by which purity improved.

**66.** (C) Apparent $K_m$ is unchanged, apparent $V_{\max}$ decreases
Non-competitive inhibition occurs when an inhibitor binds to a site other than the active site, affecting both the free enzyme and the enzyme-substrate complex with equal affinity. Because the inhibitor does not interfere with substrate binding, the Michaelis constant remains unchanged. However, the inhibitor reduces the catalytic efficiency of the enzyme, leading to a decrease in the apparent maximum velocity that cannot be overcome by adding more substrate.

**67.** (A) Lowering the dielectric constant to promote protein attraction.
Adding organic solvents like ethanol or acetone to an aqueous protein solution reduces the dielectric constant of the medium. This reduction weakens the hydration shell around protein molecules and increases the strength of electrostatic attractions between oppositely charged groups on different proteins. These enhanced attractive forces lead to the aggregation and subsequent precipitation of the proteins, allowing for their separation from the soluble components.

**68.** (D) Affinity - Separation by solubility.
Chromatography techniques utilize different physical and chemical properties to separate proteins. Gel filtration separates by size, ion-exchange by net charge, and hydrophobic interaction chromatography by surface hydrophobicity. Affinity chromatography is unique because it relies on the highly specific biological interaction between an enzyme and an immobilized ligand, such as a substrate or antibody, rather than a general property like solubility or bulk charge.

**69.** (C) Provides a uniform mass-to-charge ratio.
SDS-PAGE separates proteins based on their molecular mass by using the detergent sodium dodecyl sulfate. SDS binds to proteins, denaturing their three-dimensional structure and coating them with a uniform negative charge. This results in a consistent mass-to-charge ratio for all proteins in the sample. During electrophoresis, the electrical field drives the proteins through the gel matrix, where they separate strictly according to their size or chain length.

**70.** (C) Sequential conformation changes occur upon binding.
The Koshland-Némethy-Filmer, or sequential model, of allostery describes how the binding of a ligand to one subunit of a multimeric enzyme induces a conformational change in that specific subunit. This change then influences the neighboring subunits, altering their affinity for the ligand. Unlike the concerted model, the sequential model allows for the existence of intermediate states where different subunits within the same enzyme possess different conformations.

**71.** (D) Proteolytic activation
Proteolytic activation is an irreversible regulatory mechanism where an inactive zymogen, such as trypsinogen, is converted into an active enzyme through the cleavage of specific peptide bonds. This process is typically mediated by another protease. Once the inhibitory peptide is removed, the enzyme folds into its active shape. This mechanism is crucial for controlling the activity of digestive enzymes and the blood coagulation cascade.

**72.** (A) Channels intermediates between sites.
The pyruvate dehydrogenase complex is a massive multienzyme assembly that integrates multiple catalytic steps into a single unit. This organization allows for substrate channeling, where the intermediates of the reaction are passed directly from one active site to the next without being released into the surrounding solution. This increases the overall reaction efficiency, prevents the loss of intermediates, and protects them from competing metabolic pathways.

**73.** (B) They are artificially synthesized catalytic polymers.
Synzymes are synthetic molecules designed to mimic the catalytic functions of natural enzymes. These are often non-protein polymers or supramolecular assemblies that incorporate functional groups capable of stabilizing

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transition states or facilitating chemical transformations. Unlike naturally occurring enzymes or ribozymes, synzymes are entirely artificial and are used in research to understand the fundamental principles of catalysis and for industrial applications where natural enzymes are unstable.

**74.** (B) Coenzyme A
Coenzyme A is a vital metabolic cofactor that serves as a mobile carrier for acyl groups, such as the acetyl group in acetyl-CoA. It forms a high-energy thioester bond with these groups, allowing them to be transferred between different enzymes in pathways like the citric acid cycle and fatty acid metabolism. This mobility is essential for transporting chemical groups throughout various compartments of the cell.

**75.** (D) II and IV
Enzyme classification is based on the specific type of reaction catalyzed. Kinases are transferases because they move phosphate groups, while peptidases are hydrolases because they cleave peptide bonds using water. Phosphatases are also hydrolases, not ligases, as they remove phosphate groups via hydrolysis. Decarboxylases are lyases, as they remove carbon dioxide to form double bonds, rather than performing oxidation-reduction reactions typical of class EC 1.

**76.** (C) Providing specificity and orientation.
The binding site of an enzyme is the region responsible for recognizing and recruiting the specific substrate. It utilizes multiple weak, non-covalent interactions to provide the necessary specificity, ensuring that only the correct molecule is bound. Furthermore, the binding site aligns the substrate in the optimal orientation relative to the catalytic residues, which is critical for reducing entropy and facilitating the chemical transformation.

**77.** (D) Active site competence follows substrate binding.
According to the induced-fit hypothesis, the enzyme's active site is not perfectly complementary to the substrate in the absence of binding. Instead, the initial interaction with the substrate triggers a conformational change in the enzyme. This adjustment leads to the full assembly of the catalytic center and the precise alignment of functional groups, ensuring that the enzyme becomes catalytically competent only when the substrate is present.

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**78.** (C) Tight binding of the transition state.
Transition state theory explains that enzymes achieve massive rate enhancements by evolving active sites that are most complementary to the reaction's high-energy transition state. By binding the transition state more tightly than the ground state substrate, the enzyme stabilizes the intermediate and significantly lowers the activation energy required for the reaction. This stabilization is the primary driver of the catalytic power observed in biological systems.

**79.** (B) Proton transfers via amino acid chains.
General acid-base catalysis involves the transfer of protons to or from the substrate by functional groups on the enzyme's amino acid side chains. Histidine, aspartate, and glutamate residues often act as proton donors or acceptors during the catalytic cycle. This differs from specific acid-base catalysis, which relies solely on the concentration of hydronium or hydroxyl ions from the surrounding aqueous solvent.

**80.** (A) P-3, Q-4, R-2, S-1
Kinetic data can be visualized using various linear transformations. The Lineweaver-Burk plot uses double reciprocals of velocity and substrate concentration. The Eadie-Hofstee plot graphs velocity against the velocity-to-substrate ratio. The Hanes-Woolf plot utilizes the substrate concentration divided by velocity versus substrate concentration. Finally, the Dixon plot is specifically used to determine the inhibition constant by plotting the reciprocal of velocity against the inhibitor concentration.

**81.** (D) Reflects both catalysis and affinity.
The ratio of the turnover number, $k_{cat}$, to the Michaelis constant, $K_m$, is known as the specificity constant or catalytic efficiency. This parameter provides a comprehensive measure of how well an enzyme works at low substrate concentrations. It accounts for both the rate at which the enzyme converts the complex to product and the efficiency with which the enzyme captures the substrate from the surrounding environment.

**82.** (B) A specific ternary complex forms before any products are released.
In an ordered sequential bi-bi reaction, all substrates must bind to the enzyme in a specific, mandatory sequence before any product can be released. This results in the formation of a temporary ternary complex consisting of the enzyme and both substrates. Once the catalytic transformation occurs within this complex, the products are then released in a defined order, distinguishing this mechanism from double-displacement pathways.

**83.** (A) Parallel lines on a Lineweaver-Burk plot
Uncompetitive inhibition is uniquely identified on a Lineweaver-Burk plot by the presence of parallel lines at different inhibitor concentrations. This occurs because the inhibitor binds only to the enzyme-substrate complex, reducing both the maximum velocity and the apparent Michaelis constant by the same factor. The constant ratio between these two parameters ensures that the slopes of the lines remain identical, shifting the intercepts instead.

**84.** (D) Activation by the enzyme's catalytic mechanism.
A suicide inhibitor is a specialized type of irreversible inhibitor that is chemically unreactive until it enters the enzyme's active site. The enzyme's own catalytic mechanism then processes the molecule, converting it into a highly reactive intermediate. This newly formed species then reacts with a nearby functional group to form a stable covalent bond, effectively causing the enzyme to "commit suicide" by inactivating itself.

**85.** (B) II, III and V
The optimal pH for an enzyme's activity is critical for maintaining the correct ionization state of catalytic residues and the overall stability of the protein. Deviations from this pH can alter the protonation of functional groups in the active site or on the substrate itself, disrupting catalysis. Furthermore, extreme pH values can lead to irreversible denaturation, although enzymes typically exhibit a narrow rather than a broad pH optimum.

**86.** (C) It handles reactions without optical changes.
Discontinuous assays are particularly useful when the enzymatic reaction does not result in a direct change in light absorbance or fluorescence. In these cases, the reaction is stopped at specific time intervals, and the amount of product formed is subsequently measured using other techniques, such as chromatography, radioactivity, or chemical titration. This allows for the study of a wider variety of chemical transformations that are optically silent.

**87.** (D) Specific activity increased 50-fold.
The purification fold is a metric used to evaluate the success of an isolation procedure. It is calculated by dividing the specific activity of the final purified sample by the specific activity of the initial crude extract. A purification fold of 50 indicates that the proportion of the target enzyme relative to the total protein has increased fifty times, representing a significant improvement in purity.

**88.** (A) Bacterial peptidoglycan cell walls.
Lysozyme is an enzyme used in biological research to disrupt bacterial cells by specifically hydrolyzing the glycosidic bonds in peptidoglycan. Peptidoglycan is the primary structural component of the bacterial cell wall. By breaking these bonds, lysozyme weakens the wall, making the cells highly susceptible to osmotic lysis. This enzymatic method is genter than many physical techniques, helping to preserve the integrity of intracellular proteins.

**89.** (C) High solubility and stabilization of proteins.
Ammonium sulfate is the most commonly used salt for protein precipitation because it is highly soluble in water and does not typically denature proteins. On the contrary, high concentrations of ammonium sulfate often stabilize the native structure of proteins. This allows for bulk purification through salting-out, where different proteins precipitate at characteristic salt concentrations, facilitating their separation from the crude mixture while maintaining their biological activity.

**90.** (A) High salt to enhance interactions.
Hydrophobic interaction chromatography separates proteins based on the hydrophobic patches on their surfaces. Loading the protein onto the column in a high-salt buffer promotes these interactions by enhancing the hydrophobic effect and reducing the hydration of the protein's surface. As the salt concentration is subsequently decreased during elution, the proteins with weaker hydrophobic properties are released

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first, followed by those with more extensive hydrophobic regions.

**91.** (A) Isoelectric point in a pH gradient
Isoelectric focusing is a high-resolution electrophoretic technique that separates proteins based on their individual isoelectric points. The process occurs within a gel containing a stable pH gradient. When an electrical field is applied, each protein migrates until it reaches the specific region where the pH equals its pI. At this point, the protein has no net charge and stops moving, effectively focusing into a sharp band.

**92.** (B) The standard hyperbolic Michaelis-Menten curve
While most simple enzymes display a hyperbolic relationship between substrate concentration and reaction velocity, allosteric enzymes exhibit a sigmoidal, or S-shaped, curve. This deviation occurs because allosteric enzymes consist of multiple subunits that exhibit cooperativity. The binding of a substrate to one subunit influences the affinity of the remaining subunits, leading to a more complex kinetic profile that allows for precise regulation of metabolic pathways.

**93.** (B) One kinase activates many downstream enzymes.
Phosphorylation cascades are powerful regulatory mechanisms that allow a small initial signal to produce a massive cellular response. This is achieved through signal amplification, where a single active kinase enzyme can phosphorylate and activate numerous molecules of a second kinase. Each of these, in turn, activates many more downstream targets. This exponential increase in activity ensures that biological signals are rapidly and effectively propagated throughout the cell.

**94.** (C) Improves efficiency via substrate channeling.
Multienzyme complexes like fatty acid synthase integrate multiple catalytic domains into a single polypeptide or stable assembly. This architectural arrangement facilitates substrate channeling, where the product of one catalytic step is directly transferred to the active site of the next enzyme in the sequence. This prevents the loss of intermediates to the surrounding cytoplasm, reduces competition from other pathways, and significantly increases the overall rate and efficiency of biosynthesis.

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**95.** (A) III, I, IV, II
The catalytic cycle of a single-substrate enzyme-catalyzed reaction begins with the diffusion of the substrate molecule toward the enzyme's active site. Once the substrate enters the site, it binds to form the enzyme-substrate complex. The chemical transformation then occurs, resulting in the formation of an enzyme-product complex. Finally, the product is released into the surrounding medium, and the original enzyme is regenerated for another round of catalysis.

**96.** (A) Raising antibodies against transition-state analogs.
The development of an abzyme involves the use of immunological techniques to create a catalyst. Since antibodies naturally bind to their targets with high affinity, researchers raise antibodies against stable molecules that mimic the transition state of a chemical reaction. Because the resulting antibody active site is complementary to the transition state, it can stabilize the real intermediate of the reaction, thereby functioning as an artificial enzyme.

**97.** (A) They are tightly bound to the enzyme.
Prosthetic groups are a category of cofactors characterized by their permanent and tight association with an enzyme. Unlike soluble coenzymes that dissociate after each reaction cycle, prosthetic groups remain bound, often via covalent or very strong non-covalent bonds. They are essential for the catalytic function and structural integrity of many proteins, such as the heme group in hemoglobin or FAD in various redox enzymes.

**98.** (B) Withdraw electron density to facilitate bond breaking.
In metalloenzymes, metal ions often function as powerful electrophiles within the active site. Due to their positive charge, they can coordinate with the substrate to withdraw electron density from specific chemical bonds. This polarization makes those bonds more susceptible to nucleophilic attack or cleavage. Additionally, metal ions can orient the substrate and stabilize negatively charged intermediates, significantly contributing to the overall rate enhancement of the reaction.

**99.** (A) EC 5 (Isomerases)
Isomerases, classified under the fifth major enzyme class, specialize in catalyzing structural rearrangements within a single molecule.

These reactions do not involve the addition or removal of atoms but rather the conversion of a molecule into its geometric or structural isomer. Examples include the interconversion of glucose-6-phosphate and fructose-6-phosphate or the racemization of amino acids, which are fundamental steps in many metabolic pathways.

**100.** (D) Histidine
Histidine is uniquely suited for acid-base catalysis in biological systems because the $pK_a$ of its imidazole side chain is approximately 6.0, which is close to the physiological pH of most cells. This property allows histidine to easily alternate between protonated and deprotonated states, functioning as both a proton donor and a proton acceptor. Consequently, histidine is frequently found in the active sites of many enzymes, participating in critical proton transfer steps.

**101.** (B) Conformational selection
The conformational selection model suggests that enzymes are not static structures but rather exist in a dynamic ensemble of different shapes. Substrates bind preferentially to the specific conformation that is already complementary to their structure. This binding then shifts the population of enzyme molecules toward that active form. This view contrasts with the induced-fit model, which implies that the substrate forces a change in an initially non-complementary enzyme.

**102.** (C) Binding energy from weak interactions.
The stabilization of the transition state is primarily driven by the binding energy released when multiple weak, non-covalent interactions form between the enzyme and the high-energy intermediate. These interactions, including hydrogen bonds, ionic attractions, and Van der Waals forces, are maximized in the transition state. This specific binding provides the thermodynamic driving force necessary to lower the activation energy, enabling the reaction to proceed much more rapidly than the uncatalyzed version.

**103.** (D) Covalent catalysis
Covalent catalysis occurs when a nucleophilic group on an enzyme's amino acid side chain donates a pair of electrons to the substrate. This results in the formation of a transient covalent bond, creating a reaction intermediate that is more easily converted to the final product. By providing an alternative chemical pathway with different transition states, covalent catalysis significantly lowers the overall activation energy and increases the speed of the enzymatic reaction.

**104.** (A) Dissociation rate exceeds catalytic rate.
The Michaelis constant, $K_m$, serves as an approximate measure of the affinity between an enzyme and its substrate under specific conditions. Specifically, when the rate of dissociation of the enzyme-substrate complex back into free enzyme and substrate is much faster than the rate of product formation, $K_m$ simplifies to the dissociation constant. In this scenario, a lower $K_m$ indicates a higher affinity, meaning the enzyme binds the substrate more effectively.

**105.** (A) I and III
Establishing the purity of a protein involves multiple analytical techniques. A common criterion is the appearance of a single, sharp band on an SDS-PAGE gel, indicating the absence of contaminating proteins of different masses. Additionally, the presence of a single, symmetrical peak in size-exclusion chromatography suggests a homogeneous population of molecules. A truly pure sample should also demonstrate a maximal and constant specific activity throughout the final stages of purification.

**106.** (A) The inhibitor affects $K_m$ and $V_{\{}\max_{\}}$ to the exact same relative extent.
In uncompetitive inhibition, the inhibitor binds only to the enzyme-substrate complex, effectively reducing the concentration of the active complex available for catalysis. This results in a proportional decrease in both the maximum velocity and the Michaelis constant. Because both parameters are reduced by the same factor, the ratio of the Michaelis constant to maximum velocity, which represents the slope of the Lineweaver-Burk plot, remains constant, producing parallel lines.

**107.** (A) French press
The French press is a physical cell disruption technique that utilizes high pressure to lyse cells. A cell suspension is placed under extreme pressure and then forced through a narrow valve into a chamber at atmospheric pressure. The rapid decompression and high shear forces generated during this process cause the cell membranes and

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walls to burst, effectively releasing intracellular proteins while maintaining their native structure and biological activity.

**108.** (C) Exploit maximal binding energy.
Transition-state analogs are potent enzyme inhibitors because they are structurally designed to mimic the transition state of a chemical reaction. Because enzymes have evolved to bind the high-energy transition state with much higher affinity than the ground-state substrate, these analogs exploit the maximal binding energy available in the active site. This exceptionally strong binding effectively blocks the site, preventing natural substrate from entering and reacting.

**109.** (C) 2.0
The $Q_{\{10\}}$ value represents the factor by which a reaction rate increases for every ten-degree Celsius rise in temperature. If an enzymatic reaction's rate is observed to double when the temperature is raised from 20°C to 30°C, the $Q_{\{10\}}$ is precisely 2.0. This mathematical relationship is commonly used to describe the thermal sensitivity of biological systems, reflecting the increased kinetic energy and frequency of molecular collisions.

**110.** (A) Both A and R are true and R is the correct explanation of A.
Adding high concentrations of salt to a protein solution typically results in a decrease in solubility, a process known as salting-out. The salt ions possess a high charge density and strongly attract water molecules for hydration. This competition for solvent effectively strips the protective water layer from the protein surfaces. Consequently, hydrophobic regions on the proteins become exposed and aggregate, leading to the precipitation of the protein from the solution.

**111.** (D) Final total activity to initial total activity
The yield of an enzyme purification protocol is the percentage of the initial total enzymatic activity that is recovered in the final purified sample. It is calculated by taking the ratio of the final total activity to the starting total activity in the crude extract. While some activity is inevitably lost during each purification step, a high yield is desirable to ensure that a sufficient amount of active enzyme is obtained for downstream applications.

**112.** (D) It uses specific biological ligand interactions.
Affinity chromatography is often the most selective step in a purification protocol because it exploits the unique biological recognition between an enzyme and a specific ligand. Unlike other methods that separate proteins based on general physical properties like size or charge, affinity chromatography relies on specific binding sites. This high degree of selectivity allows for the isolation of the target protein from a complex mixture with very high purity and yield.

**113.** (A) Salt removal and buffer exchange.
Dialysis is a common technique used in protein purification to remove small molecules, such as salts or excess ligands, and to change the buffer environment. A protein solution is placed inside a semi-permeable membrane bag and submerged in a large volume of the desired buffer. Small solutes diffuse across the membrane until an equilibrium is reached, while the large protein molecules are retained inside the bag due to their size.

**114.** (C) Polyacrylamide gel for electrophoresis
Zymography is performed by incorporating a specific enzyme substrate, such as gelatin or casein, directly into a polyacrylamide gel matrix during polymerization. Proteins are then separated by electrophoresis within this substrate-enriched gel. Following the run, the gel is incubated in a buffer that allows the separated enzymes to regain activity and digest the embedded substrate. Staining subsequently reveals clear bands of activity against a dark, undigested background.

**115.** (B) II and III
Allosteric regulation involves the binding of effector molecules to specific regulatory sites that are distinct from the catalytic active site. This binding induces conformational changes in the enzyme's structure, which in turn alter its catalytic activity. These enzymes typically exhibit sigmoidal kinetics rather than hyperbolic ones, reflecting the cooperative interactions between their multiple subunits and providing a sensitive mechanism for responding to changes in metabolite concentrations.

**116.** (D) Provides signal amplification and control.
Regulatory cascades involving zymogen activation are highly advantageous for biological systems because they allow for rapid signal

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amplification. A small number of active upstream protease molecules can quickly activate a much larger number of downstream zymogens. This sequential activation ensures a massive and rapid physiological response to a stimulus, such as blood clotting or digestion, while also providing multiple points for precise control and inhibition of the pathway.

**117.** (C) 5
Lactate dehydrogenase is a tetrameric enzyme that can be composed of two different types of subunits, denoted as H and M. The random assembly of these four subunits results in exactly five distinct isoenzyme combinations: H4, H3M1, H2M2, H1M3, and M4. These isoenzymes possess different kinetic properties and are expressed in a tissue-specific manner, allowing organs to adapt their metabolic pathways to specific physiological conditions.

**118.** (C) All enzymes are exclusively made of amino acids.
While the majority of enzymes are proteins composed of amino acid chains, it is incorrect to state that all enzymes share this composition. Ribozymes are a class of catalytic RNA molecules that facilitate essential chemical reactions within cells. Furthermore, synthetic synzymes and catalytic antibodies also demonstrate enzyme-like activity. The defining characteristic of an enzyme is its ability to accelerate reactions, regardless of whether its structural framework is based on proteins or nucleic acids.

**119.** (D) Pyridoxal phosphate (PLP)
Pyridoxal phosphate, derived from vitamin B6, is a versatile coenzyme essential for all transamination reactions. It acts as a temporary carrier for amino groups by forming a covalent Schiff base intermediate with the substrate amino acid. During the catalytic cycle, the amino group is transferred from the substrate to PLP, forming pyridoxamine phosphate, which then donates the amino group to an α-keto acid to complete the reaction.

**120.** (A) Zinc - Carbonic anhydrase
Carbonic anhydrase is a classic example of a metalloenzyme that contains a tightly bound zinc ion at its active site. The zinc ion is essential for catalysis, as it coordinates with a water molecule and polarizes it to facilitate the nucleophilic attack on carbon dioxide. Without this metal ion, the enzyme is completely inactive. This specific requirement for zinc is a hallmark of the enzyme's structural and functional identity.

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**121.** (B) Peptide bonds
Enzymes in the EC 3.4 subclass are a specific group of hydrolases known as peptidases or proteases. Their primary biological function is to catalyze the hydrolytic cleavage of peptide bonds, which link amino acids together in proteins. These enzymes play vital roles in processes ranging from general protein digestion and turnover to highly specific regulatory events like the activation of zymogens or the processing of hormone precursors.

**122.** (B) Bulky aromatic side chains
The active site of chymotrypsin contains a specialized hydrophobic specificity pocket that is ideally shaped to accommodate bulky, aromatic amino acid side chains, such as those found in phenylalanine, tyrosine, and tryptophan. This pocket ensures that the enzyme cleaves peptide bonds specifically on the carboxyl side of these large hydrophobic residues. The precise dimensions and chemical nature of the pocket are key determinants of the enzyme's substrate selectivity.

**123.** (A) Transition state stabilization.
The lock-and-key model posits a rigid complementarity between the enzyme and the substrate ground state. However, this model is fundamentally flawed because an enzyme that binds its substrate perfectly would actually stabilize the ground state, thereby increasing the activation energy and slowing the reaction. Modern enzymology recognizes that enzymes must instead be complementary to the high-energy transition state to effectively lower the energy barrier and accelerate catalysis.

**124.** (C) Activation energy ($\Delta G^{\dagger}$)
Activation energy is the thermodynamic quantity that represents the energy difference between the ground state of the reactants and the highest energy point on the reaction pathway, known as the transition state. This value determines the rate at which a chemical reaction proceeds. Catalysts like enzymes function by providing a different reaction mechanism that possesses a lower activation energy, thereby allowing more molecules to react per unit time.

**125.** (B) II and IV
Enzyme inhibition mechanisms are diverse. Competitive inhibitors bind to the free enzyme at the active site, while uncompetitive inhibitors bind only to the enzyme-substrate complex. Non-competitive inhibitors bind at a separate site and do not prevent substrate binding, although they do inhibit catalysis. Suicide inhibitors are specialized molecules that undergo partial catalytic processing before forming a permanent covalent bond with the enzyme, leading to its irreversible inactivation.

**126.** (C) Covalent catalysis
A Schiff base is a common transient intermediate formed during covalent catalysis, particularly in reactions involving amino group transfers or decarboxylations. It involves the formation of a double bond between a nitrogen atom from an amino acid side chain, often lysine, and a carbonyl group on the substrate. This covalent linkage serves to stabilize negative charges and facilitate the movement of electrons, effectively lowering the activation energy of the overall reaction.

**127.** (B) Initial product concentration is zero.
A key assumption in deriving the Michaelis-Menten equation is that the kinetic measurements are taken at the very beginning of the reaction, when the product concentration is essentially zero. This ensures that the reverse reaction, where product is converted back into substrate, is negligible and does not interfere with the initial velocity. This simplification allows for a more straightforward mathematical description of the relationship between substrate concentration and reaction rate.

**128.** (D) In the second quadrant at $-K_i$.
In a Dixon plot for competitive inhibition, the reciprocal of the reaction velocity is plotted against the inhibitor concentration at different fixed substrate concentrations. The resulting lines for various substrate levels will intersect in the second quadrant of the graph. The x-coordinate of this intersection point provides a direct measurement of the negative value of the inhibition constant, $K_i$, representing the affinity of the inhibitor for the free enzyme.

**129.** (A) Multi-substrate kinetic mechanisms.
Cleland's notation is a standard shorthand system used in biochemistry to represent and visualize the complex kinetic mechanisms of multi-substrate and multi-product enzymatic reactions. It utilizes lines and arrows to denote the order in which substrates bind to the enzyme and products are released. This notation is essential for distinguishing between mechanisms such as ordered sequential, random sequential, and ping-pong double-displacement pathways.

**130.** (A) P-4, Q-1, R-2, S-3
Irreversible inhibitors are categorized by their specific mode of action. Group-specific reagents universally react with certain amino acid residues. Affinity labels are substrate mimics that possess a reactive group, targeting the active site. Suicide substrates are mechanism-based and are activated by the enzyme's own catalytic process. Transition-state analogs mimic the high-energy intermediate very closely and bind with extremely high affinity, permanently blocking the active site without necessarily forming a covalent bond.

**131.** (A) The ratio of specific activity levels.
The purification fold is a dimensionless value used to track the progress of an enzyme isolation procedure. It is calculated by dividing the specific activity of the purified sample by the specific activity of the original crude extract. An increasing purification fold indicates that the target enzyme is becoming more concentrated relative to other proteins. This metric is essential for evaluating the effectiveness of each sequential purification step.

**132.** (D) Absorbance change per unit time.
Continuous spectrophotometric assays determine the reaction rate by monitoring the change in light absorbance as a function of time. This occurs in real-time within the spectrophotometer. The slope of the resulting absorbance-versus-time curve is proportional to the enzyme's activity. This method is highly efficient as it provides immediate feedback on the initial velocity and ensures that the reaction is measured while the rate remains linear.

**133.** (A) 1 micromole of substrate per minute.
The International Unit is a standard measure of enzyme activity. One IU is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate into product per minute under standardized conditions of temperature, pH, and substrate concentration.

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While the Katal is the SI unit, the IU remains widely used in clinical and research laboratories due to its convenient scale for most biochemical experiments.

**134.** (D) Sucrose buffer followed by distilled water.
Osmotic shock is a gentle method used to disrupt the outer membranes of certain cells, particularly bacteria. Cells are first equilibrated in a high-concentration sucrose buffer, which causes them to lose water and shrink. They are then rapidly transferred to distilled water or a low-osmolarity buffer. The sudden influx of water causes the cells to swell and burst, releasing proteins, especially those located in the periplasmic space.

**135.** (A) I, II and IV
Affinity chromatography is a powerful purification technique that utilizes a specific ligand covalently attached to a solid matrix. The target enzyme binds selectively to the ligand while other proteins pass through. The enzyme is subsequently eluted by adding a solution containing a high concentration of the free ligand or by altering the pH. This method can achieve very high purification folds in a single step due to its extreme selectivity.

**136.** (A) Preserve complexes and activity.
Native-PAGE is chosen over SDS-PAGE when it is necessary to preserve the protein's original three-dimensional structure and biological activity. Because it omits denaturing detergents and reducing agents, multisubunit complexes remain intact, and enzymes often retain their catalytic power. This allows for the analysis of the protein's native state, including its oligomeric composition and its ability to function after being separated from other components in the mixture.

**137.** (B) $\log\left(\frac{v}{V_{\max} - v}\right)$ versus $\log[S]$
The Hill plot is a graphical tool used to determine the Hill coefficient, which quantifies the degree of cooperativity in an allosteric enzyme. By plotting the log of the ratio of fractional occupancy to unoccupied sites against the log of the substrate concentration, a straight line is obtained. The slope of this line represents the Hill coefficient. A slope greater than one indicates positive cooperativity among the enzyme subunits.

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**138.** (B) ATP
Protein kinases are essential regulatory enzymes that catalyze the transfer of a phosphate group from a donor molecule to a specific amino acid residue on a target protein. In physiological systems, adenosine triphosphate (ATP) serves as the universal phosphate donor. The kinase binds both ATP and the substrate protein, facilitating the nucleophilic attack of the protein's hydroxyl group on the terminal gamma-phosphate of the ATP molecule.

**139.** (A) Electrophoretic mobility
Isoenzymes are distinct molecular forms of the same enzyme that catalyze the identical chemical reaction. Due to differences in their amino acid sequences, they typically possess different net electrical charges. This allows them to be accurately separated and differentiated in the laboratory based on their unique electrophoretic mobilities. When placed in an electrical field, each isoenzyme migrates at a characteristic rate, producing a specific pattern of bands in a gel.

**140.** (C) Hexokinase closure upon glucose binding.
A classic piece of evidence for the induced-fit model is the behavior of the enzyme hexokinase. X-ray crystallography has revealed that the enzyme undergoes a major conformational change when it binds to its substrate, glucose. The two lobes of the enzyme close around the glucose molecule, bringing the catalytic residues into the correct position and excluding water from the active site. This dynamic adjustment is essential for efficient catalysis.

**141.** (D) Product concentration versus time at $t \approx 0$
The initial velocity of an enzymatic reaction is determined by measuring the rate of product formation at the very beginning of the reaction, before the substrate is significantly depleted. This is achieved by calculating the slope of a graph plotting product concentration against time near the starting point. Using initial velocity ensures that the measurement is not affected by product inhibition, substrate exhaustion, or the reverse reaction during the assay.

**142.** (C) Lowers functional enzyme concentration.
Non-competitive inhibitors bind to a site other than the active site, affecting both the free enzyme and the enzyme-substrate complex. This binding reduces the overall catalytic efficiency of the enzyme population, effectively lowering

and release proteins that are bound to the stationary phase.

**176.** (D) Metal-activated enzymes
Metal-activated enzymes require the presence of specific metal ions, such as potassium or magnesium, to achieve their full catalytic potential. These ions are often loosely associated with the enzyme and can be easily removed or exchanged. Unlike metalloenzymes, which have tightly bound metal centers that are permanent structural features, metal-activated enzymes rely on the metal concentration in the surrounding medium to stabilize substrates or facilitate the chemical reaction during the catalytic cycle.

**177.** (A) Hydrolases (EC 3)
Proteases are a diverse group of enzymes categorized as hydrolases under the International Union of Biochemistry and Molecular Biology classification system. Their primary function is to catalyze the hydrolysis of peptide bonds in proteins and peptides. By adding a water molecule across the bond, they break the polypeptide chain into smaller fragments. These enzymes are essential for protein digestion, cellular protein turnover, and the precise regulation of various metabolic pathways.

**178.** (C) Aspartate, Histidine, Serine
The catalytic triad of chymotrypsin is a highly conserved arrangement of three amino acids: aspartate, histidine, and serine. These residues work in a coordinated manner to facilitate the hydrolysis of peptide bonds. Serine acts as the nucleophile that attacks the substrate, while histidine serves as a general base to activate the serine. Aspartate helps to orient the histidine and stabilize the positive charge that develops during the reaction, enabling efficient catalysis.

**179.** (A) Distorted reaction transition states.
According to transition state theory, enzymes have evolved to possess the highest binding affinity for the high-energy, distorted transition state of a chemical reaction. By preferentially binding and stabilizing this intermediate over the ground-state substrate, the enzyme lowers the activation energy required for the reaction to proceed. This specific complementarity is the fundamental source of the catalytic power and rate enhancement observed in all biological enzyme systems.

**180.** (D) Offset the energy cost of transition states.
Binding energy is the free energy released when an enzyme forms multiple weak, non-covalent interactions with its substrate. This energy is not used to change the overall thermodynamics of the reaction but is specifically employed to offset the high energy cost associated with reaching the transition state. By providing this stabilizing energy, enzymes effectively lower the activation barrier, allowing the chemical transformation to occur much more rapidly and efficiently.

**181.** (B) Stabilizing by charge distribution.
Electrostatic catalysis accelerates enzymatic reactions by utilizing specific arrangements of charged amino acid side chains within the active site. These charges create a local electrical field that stabilizes polar or charged transition states. By optimally distributing charge and excluding bulk water, the enzyme reduces the energy required to reach the transition state. This mechanism is particularly important in reactions involving the formation or breakdown of charged intermediates and dipoles.

**182.** (C) Shifting the curve to the right, increasing the apparent $K_m$.
In a Michaelis-Menten plot, a competitive inhibitor shifts the hyperbolic velocity-versus-substrate curve to the right. This shift indicates an increase in the apparent Michaelis constant, as more substrate is required to achieve the same velocity in the presence of the inhibitor. However, because competitive inhibition can be overcome by high substrate concentrations, the curve eventually reaches the same maximum velocity as the uninhibited reaction, given enough substrate.

**183.** (A) I and II
A classic coupled enzyme assay involves monitoring two separate but linked chemical reactions. The primary reaction is the one being studied, and its progress is tracked by the second, auxiliary reaction. The measure of interest is the rate of change in a detectable component of the auxiliary reaction, such as the appearance or disappearance of a fluorescent or light-absorbing molecule. This allow for the quantification of the primary enzyme's catalytic activity.

**184.** (A) Parallel lines.
In multi-substrate ping-pong, or double-displacement mechanisms, the Lineweaver-Burk

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that can stabilize transition states and accelerate reactions. Their existence suggests that in early evolution, RNA may have served both as a carrier of genetic information and as the primary catalyst for biochemical transformations in a hypothetical "RNA world."

**151.** (D) Coenzyme
A coenzyme is a small organic molecule that is loosely associated with an enzyme and is necessary for its catalytic activity. Coenzymes often function as intermediate carriers of chemical groups, such as electrons, protons, or specific functional groups like acyl or methyl units. They frequently participate in the reaction and then dissociate from the enzyme to be regenerated elsewhere, facilitating the transfer of chemical entities between different metabolic pathways.

**152.** (A) Require environmental metal activation.
Metal-activated enzymes are characterized by their requirement for specific metal ions that are loosely and reversibly associated with the protein. These ions are often acquired from the surrounding cellular environment or buffer and can be easily lost during purification. This contrasts with metalloenzymes, which contain metal ions that are tightly and permanently integrated into the protein structure, remaining associated throughout the life of the enzyme molecule.

**153.** (C) Hydrolysis of ATP phosphate bonds.
Ligases, belonging to enzyme class EC 6, catalyze the formation of new chemical bonds, such as C-C, C-S, C-O, or C-N bonds, by joining two large molecules. Because bond formation is an endergonic process, these reactions are typically coupled to the exergonic hydrolysis of a high-energy phosphate bond, usually from ATP. This energy coupling provides the necessary thermodynamic driving force to enable the synthesis of complex biological molecules.

**154.** (A) To distinguish and bind correct structures.
The active site of an enzyme includes a specialized binding pocket lined with amino acid side chains that determine the enzyme's specificity. These residues utilize a combination of shape complementarity and weak, non-covalent interactions, such as hydrogen bonds and hydrophobic forces, to selectively recognize and bind the correct substrate molecule. By excluding incorrect molecules, the specificity pocket ensures that the enzyme catalyzes only the intended biochemical reaction.

**155.** (A) I and II
Linear transformations of the Michaelis-Menten equation are useful for visualizing kinetic data. However, the Lineweaver-Burk plot is statistically flawed because it places disproportionate weight on data collected at low substrate concentrations, where relative error is often highest. In contrast, the Hanes-Woolf plot provides a more even distribution of data points and minimizes the impact of these errors, making it a more robust method for determining kinetic constants from experimental results.

**156.** (C) The free energy of activation.
The free energy of activation is the specific amount of energy that must be overcome for a chemical reaction to occur. It represents the difference in energy between the ground state of the reactants and the high-energy transition state. In biological systems, enzymes function as catalysts by providing an alternative reaction pathway that possesses a lower free energy of activation, thereby allowing the reaction to proceed much more quickly at body temperature.

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**157.** (D) Abstracting a proton for nucleophilicity.
A general base catalyst enhances reaction rates by abstracting a proton from a substrate or a neighboring molecule, such as water. This removal of a proton increases the nucleophilicity of the molecule, making it more reactive and better able to attack other functional groups. Histidine is a common residue that acts as a general base in many enzymatic mechanisms due to the appropriate $pK_a$ of its imidazole side chain.

**158.** (C) $k_{cat} = \frac{V_{\max}}{[E_t]}$
The turnover number, $k_{\{cat\}}$, is a fundamental kinetic constant that represents the maximum number of substrate molecules converted to product by a single enzyme active site per unit of time. It is calculated by dividing the maximum velocity of the reaction, $V_{\max}$, by the total concentration of enzyme active sites, $E_t$ . This value allows for the direct comparison of catalytic power between different enzymes, independent of their concentrations.

side chain, forming a stable covalent bond that permanently blocks the catalytic function. Because this residue is critical for the mechanism of serine proteases and acetylcholinesterase, DIPF is highly toxic and is used extensively to map active sites.

**210.** (A) Both A and R are true and R is the correct explanation of A.
Heavy metal ions, such as mercury and lead, are powerful irreversible inhibitors of many enzymes. They exert their toxic effects by binding with high affinity to free sulfhydryl groups on cysteine residues. This binding can directly interfere with the catalytic mechanism or disrupt the critical disulfide bonds that maintain the protein's native three-dimensional structure. Consequently, the enzyme becomes permanently denatured or inactivated, leading to severe disruptions in cellular metabolism.

**211.** (A) Monitoring real-time absorbance changes.
Continuous spectrophotometric assays provide a real-time record of enzymatic activity by measuring changes in light absorbance as the reaction occurs. This method is highly efficient because it allows the researcher to immediately calculate the initial velocity from the slope of the absorbance-versus-time curve. It also ensures that the reaction is monitored while the rate remains linear, providing more accurate and reliable kinetic data than methods that require stopping the reaction at intervals.

**212.** (D) Total enzymatic activity remaining to initial total enzymatic activity.
The percentage yield is an important metric used to evaluate the efficiency of an enzyme purification protocol. it represents the proportion of the starting total enzymatic activity that is recovered after each purification step. While some activity is always lost during isolation, a high yield is necessary to ensure that a sufficient amount of the target enzyme is obtained for downstream applications. This value is calculated by dividing final total activity by initial total activity.

**213.** (B) Bacterial periplasmic space.
Osmotic shock is a gentle physical disruption technique that is particularly useful for isolating proteins from the periplasmic space of gram-negative bacteria. By first shrinking the cells in a high-concentration sucrose buffer and then rapidly transferring them to a low-osmolarity buffer, the outer membrane is selectively compromised. This allows for the release of periplasmic proteins without the extensive release of cytoplasmic contents, simplifying the initial steps of the purification process.

**214.** (C) Competition for water leading to aggregation.
Salting-out is a fundamental principle of protein purification where high concentrations of neutral salts, such as ammonium sulfate, lead to the precipitation of proteins. The salt ions attract water molecules for hydration, effectively competing with the protein molecules for the available solvent. As the protein's hydration shell is reduced, hydrophobic patches on their surfaces are exposed and interact with each other. This leads to the formation of large aggregates that precipitate out of solution.

**215.** (D) Specific biological ligand interactions.
Affinity chromatography is a highly selective purification method that relies on the unique biological interaction between an enzyme and a specific ligand. The ligand, which could be a substrate analog, cofactor, or antibody, is covalently attached to a stationary phase. As the crude mixture passes through, only the target enzyme binds to the ligand. This specific recognition allows for the isolation of the protein with very high purity and yield in a single step.

**216.** (A) Applying a uniform negative charge per unit mass.
SDS-PAGE ensures that proteins separate based on their molecular mass by utilizing the detergent sodium dodecyl sulfate. SDS denatures the protein's native structure and binds uniformly along the polypeptide chain, imparting a consistent negative charge per unit of mass. This results in all proteins having a similar charge-to-mass ratio. During electrophoresis, the gel matrix acts as a molecular sieve, allowing smaller proteins to migrate faster than larger ones through the electrical field.

**217.** (B) Sigmoidal curve
Allosteric enzymes are characterized by their complex subunit interactions, which lead to a sigmoidal, or S-shaped, relationship between substrate concentration and reaction velocity. This deviates from the hyperbolic curve seen in traditional Michaelis-Menten kinetics. The sigmoidal shape reflects cooperativity, where the

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involves subjecting a cell suspension to extremely high pressure and then forcing it through a narrow orifice. The sudden drop to atmospheric pressure causes rapid decompression, which, combined with the intense shear forces experienced during the passage through the valve, causes the cell walls and membranes to rupture, releasing the intracellular contents into the buffer.

**168.** (A) Maintain temperature below zero.
While organic solvents like ethanol are excellent for precipitating proteins by lowering the dielectric constant, they can also cause denaturation at room temperature. To prevent the loss of native structure and biological activity, organic solvent precipitation is typically performed at very low temperatures, often below zero degrees Celsius. These cold conditions reduce the kinetic energy of the system, helping to stabilize the protein's folded state during the aggregation and precipitation process.

**169.** (A) Large proteins elute first via void volume.
In gel filtration chromatography, the stationary phase consists of porous beads. Smaller protein molecules enter these pores and are slowed down as they move through the column. Large proteins, however, are excluded from the pores because they cannot fit inside. Consequently, they travel only through the spaces between the beads, following the shortest path through the void volume. This leads to large proteins eluting first, followed by proteins of decreasing size.

**170.** (C) pH gradient
Isoelectric focusing is a high-resolution separation technique that relies on the migration of proteins within a stable pH gradient established in a gel. When an electrical field is applied, each protein moves toward the pH that corresponds to its unique isoelectric point. Once a protein reaches its pI, its net charge becomes zero, and it stops migrating. This results in the focusing of proteins into sharp, distinct bands based on their specific charge properties.

**171.** (A) Is the normal substrate at regulatory sites.
A homotropic modulator is an allosteric regulator that is identical to the enzyme's natural substrate. In these systems, the binding of a substrate molecule to one active or regulatory site induces conformational changes that influence the binding of subsequent substrate molecules at other sites. This behavior is the basis of cooperativity in allosteric enzymes, allowing for a sensitive, sigmoidal response to changes in substrate concentration within the cell.

**172.** (C) Removing phosphate by bond hydrolysis.
Protein phosphatases are essential regulatory enzymes that reverse the action of protein kinases. They catalyze the removal of phosphate groups from specific phosphorylated amino acid residues, such as serine, threonine, or tyrosine. This process occurs through the hydrolytic cleavage of the phosphoester bond, releasing inorganic phosphate and regenerating the original hydroxyl group on the protein. This reversible modification is a fundamental mechanism for controlling various cellular processes.

**173.** (B) Allow for easy enzyme recovery and reuse.
Enzyme immobilization involves attaching enzymes to a solid, insoluble support or encapsulating them within a matrix. This technique is widely used in industry because it allows the catalyst to be easily separated from the product stream, facilitating its recovery and repeated use. Immobilization often enhances the stability of the enzyme against harsh conditions, such as high temperatures or organic solvents, making industrial processes more cost-effective and efficient.

**174.** (D) Artificially synthesized catalytic polymers.
Synzymes are artificial catalysts designed by scientists to mimic the extraordinary efficiency and specificity of natural enzymes. These molecules are typically composed of non-biological polymers, such as dendrimers or functionalized resins, that incorporate specific chemical groups capable of binding substrates and stabilizing transition states. Studying synzymes helps researchers understand the fundamental principles of catalysis and allows for the creation of robust catalysts for industrial applications where natural enzymes might fail.

**175.** (A) P-2, Q-3, R-4, S-1
Chromatography elution methods vary by technique. Ion-exchange often uses an increasing salt gradient to displace bound proteins. Affinity chromatography typically requires adding a free ligand to compete for the binding site. Gel filtration is isocratic, meaning proteins elute in the same buffer without a gradient. Hydrophobic interaction chromatography relies on a decreasing salt gradient to reduce the hydrophobic effect

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**193.** (A) SDS and reducing agents.
Native-PAGE is an electrophoretic method designed to separate proteins while maintaining their original folded structure and biological activity. It achieves this by omitting denaturing agents like sodium dodecyl sulfate (SDS) and reducing agents like β-mercaptoethanol. Without these chemicals, proteins retain their native charge-to-mass ratio and multisubunit associations. This allows researchers to study active enzymes and intact protein complexes, which would otherwise be destroyed by the denaturing conditions used in SDS-PAGE.

**194.** (C) Propagating sequential changes.
The sequential or KNF model of allostery describes how the binding of a ligand to one subunit of a multimeric enzyme induces a conformational change that is restricted to that specific subunit. This change then subtly alters the interactions with neighboring subunits, sequentially affecting their affinity for subsequent ligand binding events. This model allows for a gradual transition between low and high affinity states, explaining why all subunits do not have to change shape simultaneously.

**195.** (A) IV, III, I, II
Determining the specific activity of an enzyme requires a precise sequence of laboratory steps. First, the purified enzyme sample must be obtained. Then, an enzymatic assay is performed to determine the total activity present in the sample. Separately, the total protein concentration is measured using a standard method like the Bradford assay. Finally, the specific activity is calculated by dividing the total enzymatic activity by the total mass of protein in the sample.

**196.** (D) Prevent premature enzyme activity.
Zymogens are inactive precursors of enzymes that are synthesized and stored until their activity is needed. This biological strategy is essential for preventing premature enzyme activity, which could be harmful to the cell or tissue where the enzyme is produced. For example, digestive proteases are secreted as zymogens to avoid damaging the pancreas. Activation occurs only at the final destination through specific proteolytic cleavage, ensuring activity is restricted to the appropriate physiological location.

**197.** (A) Zero-order kinetics.
When an enzyme's Michaelis constant is much lower than the physiological substrate concentration, the enzyme is virtually always saturated with substrate. Under these conditions, every active site is occupied, and the reaction velocity reaches its maximum. The rate becomes independent of any further increases in substrate concentration, a condition known as zero-order kinetics. This allows the enzyme to maintain a constant and maximal metabolic flux regardless of fluctuations in substrate availability.

**198.** (C) Complex proteinaceous or RNA molecules.
True enzymes are complex biological catalysts that are primarily composed of proteins, which are chains of amino acids folded into specific three-dimensional shapes. Additionally, certain RNA molecules known as ribozymes also exhibit significant catalytic power. These molecules create unique active site environments that stabilize transition states and accelerate biochemical reactions. Their intricate structures are essential for the high degree of specificity and efficiency that characterizes all enzymatic transformations in living organisms.

**199.** (D) Prosthetic group
A prosthetic group is a non-protein component that is tightly and permanently associated with an enzyme, often through covalent bonds. These groups are essential for the enzyme's catalytic activity and structural stability. Unlike soluble coenzymes that dissociate after each reaction, prosthetic groups remain bound throughout the enzyme's life cycle. Common examples include the heme group in various redox enzymes and certain metal ions that are integral to the active site architecture.

**200.** (A) Metalloenzyme
Carbonic anhydrase is classified as a metalloenzyme because it contains a zinc ion that is tightly and permanently coordinated within its active site. This metal ion is an absolute requirement for the enzyme's function, as it directly participates in the catalytic mechanism by coordinating with water. Because the zinc remains associated with the protein during purification and is integral to its native structure, carbonic anhydrase serves as a classic model for metalloenzyme study.

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plot shows a characteristic pattern of parallel lines when the concentration of one substrate is varied while the other is held at different fixed levels. This occurs because the enzyme exists in two different stable forms, and the substrates do not form a ternary complex. The lack of interaction between the substrate binding events results in a constant slope across the different kinetic trials.

**185.** (C) I and II
Enzymes are biological catalysts that accelerate chemical reactions by lowering the activation energy, thereby increasing both the forward and reverse reaction rates to the same extent. Critically, enzymes do not change the overall standard free energy of the reaction or the final position of the thermodynamic equilibrium. They simply allow the system to reach that equilibrium state much more quickly than would be possible in the absence of the catalyst.

**186.** (B) 5-Fluorouracil
5-Fluorouracil is a well-known suicide substrate for the enzyme thymidylate synthase, which is essential for DNA synthesis. Once inside the cell, it is converted into a reactive intermediate that binds covalently to the enzyme's active site during the catalytic cycle. This permanent modification irreversibly inactivates the enzyme, effectively blocking the production of thymine nucleotides. Because of this mechanism, 5-fluorouracil is widely used as a potent chemotherapeutic agent in cancer treatment.

**187.** (A) Salting-in
Salting-in is the phenomenon where the solubility of a protein increases as a small amount of salt is added to the solution. The salt ions interact with the charged groups on the protein's surface, effectively shielding them and reducing the electrostatic attraction between different protein molecules. This shielding helps to prevent aggregation and promotes the stabilization of the protein's native structure in an aqueous environment, enhancing its overall solubility and functionality.

**188.** (C) The enzyme or substrate.
In any enzymatic assay, a blank or control sample is essential to account for non-enzymatic changes in the measured signal, such as background absorbance. This sample is identical to the experimental reaction mixture but lacks either the enzyme or the substrate. By measuring the blank, researchers can subtract any spontaneous reaction or optical interference, ensuring that the final calculated activity reflects only the catalysis mediated by the enzyme being studied.

**189.** (A) 10
The purification fold is a quantitative measure of how much the purity of an enzyme has improved during a purification process. It is calculated by taking the ratio of the specific activity of the final sample to the specific activity of the starting material. If the specific activity increases from 10 U/mg to 100 U/mg, the purification fold is precisely 10, indicating a ten-fold increase in the enzyme's purity.

**190.** (C) Catalyst hydrolyzing peptidoglycan.
Lysozyme is a specialized enzyme that facilitates cell disruption by acting as a biological catalyst. It specifically targets and hydrolyzes the glycosidic bonds within the peptidoglycan layer of bacterial cell walls. By breaking these essential structural bonds, lysozyme weakens the cell wall's integrity. When followed by a change in osmotic pressure, the weakened cells easily burst, releasing their intracellular components into the surrounding buffer for further purification and analysis.

**191.** (B) Attraction between charged proteins.
Organic solvents like ethanol or acetone precipitate proteins by reducing the dielectric constant of the aqueous environment. A lower dielectric constant decreases the ability of the solvent to shield electrical charges. Consequently, the electrostatic attractive forces between oppositely charged groups on different protein molecules become significantly stronger. This leads to the formation of large protein aggregates that subsequently precipitate out of the solution, allowing for their easy recovery and separation.

**192.** (D) Net surface charge at a specific pH.
Ion-exchange chromatography is a widely used technique for separating proteins based on their net surface charge. The net charge of a protein is determined by the surrounding pH and its unique isoelectric point. By selecting an appropriate buffer pH and a matrix with a complementary charge, researchers can ensure that the target protein binds selectively to the chromatography column. The protein is then eluted by increasing the salt concentration or altering the pH.

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the concentration of functionally active enzyme molecules. Consequently, the maximum velocity of the reaction decreases. Because the inhibitor does not compete for the active site, the Michaelis constant remains unchanged regardless of the inhibitor concentration.

**143.** (B) Competitive can be overcome by high substrate; uncompetitive cannot.
Competitive and uncompetitive inhibition are distinguished by their kinetic behavior at high substrate concentrations. In competitive inhibition, the inhibitor and substrate vie for the same active site. Adding a vast excess of substrate can effectively displace the inhibitor, allowing the reaction to reach its original maximum velocity. However, in uncompetitive inhibition, the inhibitor binds only to the enzyme-substrate complex, so increasing the substrate concentration actually facilitates more inhibition.

**144.** (D) Lowering the dielectric constant for attraction.
Precipitation of proteins using organic solvents like ethanol works by decreasing the dielectric constant of the solvent medium. Water has a very high dielectric constant, which effectively shields charged groups on proteins from each other. When an organic solvent is added, the dielectric constant drops, allowing the electrostatic attraction between oppositely charged groups on different protein molecules to strengthen. This leads to aggregation and subsequent precipitation from the solution.

**145.** (A) Homogenization, Salt Fractionation, Ion-Exchange Chromatography, Gel Filtration
A typical enzyme purification protocol follows a logical progression from crude extraction to high-resolution separation. It begins with homogenization to disrupt cells. This is followed by salt fractionation, such as ammonium sulfate precipitation, for bulk concentration. High-capacity chromatography, like ion-exchange, is then used for further purification based on charge. Finally, gel filtration is often employed as a polishing step to separate proteins by size and to perform buffer exchange.

**146.** (C) Proteases.
Zymography is a specialized electrophoretic technique that is most frequently applied to the study of proteases. By embedding a protein substrate like gelatin or casein into a polyacrylamide gel, researchers can visualize the activity of individual protease enzymes after they have been separated by mass. Clear zones on the stained gel indicate areas where the proteases have digested the substrate, providing both molecular weight information and evidence of catalytic function.

**147.** (A) Cooperative binding of subunits.
Allosteric enzymes generally consist of multiple subunits that exhibit cooperative binding. This means that the binding of a substrate or effector to one subunit induces conformational changes that are transmitted to the other subunits, altering their affinity. This cooperativity results in sigmoidal kinetics, which differ from the standard hyperbolic curves of Michaelis-Menten kinetics. This behavior allows these enzymes to respond very sensitively to small changes in metabolite levels.

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**148.** (D) Chymotrypsinogen cleavage to active enzyme.
Proteolytic activation, such as the conversion of chymotrypsinogen to chymotrypsin, is a classic example of an irreversible regulatory mechanism. It involves the cleavage of specific peptide bonds in an inactive precursor molecule by another protease. Once these bonds are broken, the protein undergoes a permanent conformational change to its active form. This mechanism is vital for controlling enzymes that could cause cellular damage if they were active prematurely.

**149.** (C) NAD+
Nicotinamide adenine dinucleotide (NAD+) is a soluble coenzyme that acts as a mobile carrier of electrons and protons during redox reactions. Unlike prosthetic groups that remain tightly bound, NAD+ behaves more like a substrate; it binds to an enzyme, participates in the chemical transformation, and then dissociates to carry its cargo to another enzyme or metabolic pathway. This mobility is essential for connecting different metabolic processes within the cell.

**150.** (C) Are composed of RNA instead of amino acids.
Ribozymes represent a significant discovery in biochemistry because they demonstrate that catalytic activity is not restricted to proteins. These are RNA molecules that fold into complex three-dimensional shapes, creating active sites

**201.** (A) Transferases (EC 2)
Transferases are a major class of enzymes responsible for moving a specific functional group, such as a methyl, acyl, or phosphate group, from one molecule to another. These enzymes play a critical role in various metabolic pathways, including energy transfer and signal transduction. Kinases are a prominent example, as they facilitate the transfer of phosphate groups from ATP to other substrates, regulating the activity of numerous proteins and metabolites within the cell.

**202.** (A) The lock-and-key model.
The realization that enzyme active sites are complementary to the transition state rather than the ground state substrate directly refutes the traditional lock-and-key model. If an enzyme fit the ground state perfectly, it would stabilize the substrate and hinder the reaction. Instead, modern enzymology recognizes that binding energy is optimized for the transition state, which is essential for lowering the activation barrier and achieving the massive rate enhancements characteristic of biological catalysts.

**203.** (A) The activation energy.
Enzymes function as catalysts by specifically lowering the activation energy of a chemical reaction. They achieve this by providing an alternative reaction pathway that involves more stable transition states. While enzymes significantly increase the rate of both the forward and reverse reactions, they do not alter the energy levels of the reactants or products, nor do they change the overall standard free energy or the final equilibrium position of the reaction.

**204.** (C) Proton donation from active site groups.
General acid catalysis involves the transfer of a proton from a specific functional group on an enzyme's amino acid side chain to the substrate. This proton donation helps to stabilize a developing charge or facilitate the cleavage of a chemical bond. Histidine, aspartate, and glutamate are frequently involved in this process. This mechanism allows the enzyme to provide a more favorable reaction pathway than would be possible through specific acid catalysis in bulk water.

**205.** (B) II and III
The Michaelis constant, $K_m$, is a fundamental kinetic parameter defined as the substrate concentration at which the reaction velocity is half-maximal. It serves as an inverse measure of the affinity between the enzyme and its substrate; a low $K_m$ indicates a high affinity, meaning the enzyme can reach its maximum rate at lower substrate levels. Unlike maximum velocity, $K_m$ is an intrinsic property of the enzyme and does not change with enzyme concentration.

**206.** (D) $V_{\max}$
In a standard Michaelis-Menten plot of initial velocity versus substrate concentration, the curve asymptotically approaches a maximal value known as $V_{\max}$. This represents the point where all enzyme active sites are saturated with substrate. However, in linear transformations like the Eadie-Hofstee plot, the maximum velocity can be directly determined from the y-intercept. In a Lineweaver-Burk plot, the y-intercept corresponds to the reciprocal of the maximum velocity, providing a clear graphical representation.

**207.** (B) Either substrate can bind the enzyme first.
A random sequential multi-substrate mechanism is characterized by the formation of a ternary complex where both substrates bind to the enzyme before any products are released. Unlike the ordered sequential mechanism, there is no mandatory sequence for binding; either substrate can enter the active site first. This flexibility is reflected in the kinetic profile of the enzyme and is a common feature of many kinases and dehydrogenases that utilize multiple substrates in their catalytic cycles.

**208.** (D) Pure non-competitive
Pure non-competitive inhibition is identified on a Lineweaver-Burk plot by a set of lines that intersect precisely on the horizontal x-axis. This intersection point corresponds to the value of the negative reciprocal of the Michaelis constant. This pattern occurs because the inhibitor binds to both the free enzyme and the enzyme-substrate complex with equal affinity, decreasing the maximum velocity while leaving the apparent affinity for the substrate entirely unaffected.

**209.** (A) Serine
Diisopropyl fluorophosphate (DIPF) is a potent irreversible inhibitor that specifically targets and covalently modifies serine residues within the active sites of many enzymes. It reacts with the nucleophilic hydroxyl group of the serine

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**159.** (B) $-\frac{1}{K_m}$
In the Lineweaver-Burk double-reciprocal plot, the reciprocal of the reaction velocity is plotted against the reciprocal of the substrate concentration. When the resulting straight line is extrapolated to the horizontal axis, the x-intercept is equal to the negative reciprocal of the Michaelis constant. This graphical method provides a convenient way to determine $K_m$, which is an important parameter reflecting the affinity and kinetic properties of an enzyme.

**160.** (C) A is true but R is false.
The conformational selection model correctly asserts that enzymes are dynamic molecules that naturally sample multiple structural states. A substrate will preferentially bind to the specific conformation that is already complementary to its shape, shifting the population toward that active state. This contradicts the idea that enzymes are rigid; rather, their flexibility is a fundamental property that allows them to interact with substrates and effect catalysis.

**161.** (A) Analyzing specific product inhibition patterns.
Distinguishing between ordered and random sequential multi-substrate mechanisms requires detailed kinetic analysis. While both mechanisms involve the formation of a ternary complex, they differ in the mandatory or optional sequence of substrate binding. By studying product inhibition patterns, researchers can determine if the release of one product specifically interferes with the binding of a particular substrate, which reveals the degree of order or randomness in the catalytic cycle.

**162.** (C) It decreases both $V_{\max}$ and apparent $K_m$ proportionately.
Uncompetitive inhibition occurs when an inhibitor binds only to the enzyme-substrate complex. This binding removes some of the complex from the reaction, leading to a decrease in the maximum velocity. Simultaneously, the removal of the complex shifts the equilibrium to favor more substrate binding, which results in a proportional decrease in the apparent Michaelis constant. Because both parameters are reduced by the same factor, the slopes in a Lineweaver-Burk plot remain parallel.

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**163.** (A) The sulfhydryl group of cysteine.
Iodoacetamide is an irreversible group-specific reagent commonly used in protein chemistry and enzyme mapping. It specifically targets and alkylates the nucleophilic sulfhydryl groups of cysteine residues. By forming a stable covalent bond with the sulfur atom, iodoacetamide permanently modifies the cysteine side chain. This reagent is particularly useful for identifying critical cysteines in active sites or for preventing the formation of unwanted disulfide bonds during protein analysis.

**164.** (D) The $pK_a$ of critical active site groups.
The optimal pH for enzyme activity is primarily determined by the $pK_a$ values of the amino acid side chains that participate in substrate binding and catalysis. For the enzyme to be active, these groups must be in the correct protonation state. Changes in pH alter the concentration of protons in the medium, shifting the ionization of these critical residues. When the pH matches the requirements for all essential groups, the enzyme achieves its maximum rate.

**165.** (B) Difficulty in measuring rapid initial rates.
Discontinuous assays require stopping the reaction at fixed intervals to measure product formation, which makes them less suitable for studying very fast reactions. Because there is a time delay between starting the reaction and taking the first measurement, the rapid initial velocity may be missed. This is a significant disadvantage compared to continuous assays, which provide a real-time record of the reaction progress and allow for the precise determination of early kinetic rates.

**166.** (C) Most of the protein mass is the active target enzyme.
Specific activity is a measure of enzyme purity, defined as the total units of activity per milligram of total protein. A high specific activity indicates that a large proportion of the protein in the sample is the active target enzyme, with minimal contamination from other non-catalytic proteins. During purification, researchers monitor this value; an increase in specific activity confirms that the isolation steps are effectively removing unwanted proteinaceous material.

**167.** (D) Shears cells by rapid decompression.
French press disruption is a highly effective physical method for lysing tough bacterial cells. It

binding of substrate to one subunit increases the affinity of the others. This mechanism allows the enzyme to respond sensitively to small changes in substrate levels, providing efficient metabolic regulation.

**218.** (B) Covalent phosphorylation.
Regulatory cascades frequently utilize protein kinases to exert control over metabolic pathways through covalent phosphorylation. Kinases catalyze the transfer of a phosphate group from ATP to specific amino acid residues, inducing conformational changes that either activate or inhibit target enzymes. This modification is reversible, as protein phosphatases can later remove the phosphate group. This dynamic system allows for rapid and sensitive coordination of cellular activities in response to various internal and external stimuli.

**219.** (A) Intermediates stay within the complex.
Substrate channeling is a major advantage of multienzyme complexes, where the product of one catalytic step is passed directly to the active site of the next enzyme in the pathway without being released into the surrounding medium. This organization increases the local concentration of intermediates, reduces the time required for diffusion, and prevents the loss of intermediates to competing metabolic pathways. Consequently, substrate channeling significantly enhances the overall efficiency and flux of the biochemical pathway.

**220.** (C) Antibodies that bind transition states.
Abzymes, or catalytic antibodies, bridge the fields of enzymology and immunology. They are created by raising antibodies against stable molecules that mimic the high-energy transition state of a chemical reaction. Because the binding site of the antibody is designed to be complementary to this transition state, it can stabilize the real intermediate of a reaction. This stabilization lowers the activation energy, enabling the antibody to function as a specific and effective biological catalyst.

**221.** (C) Holoenzyme
A holoenzyme is the complete, functional, and catalytically active form of an enzyme. It consists of the protein component, known as the apoenzyme, along with all the necessary non-protein factors required for activity. These cofactors can include metal ions or complex organic molecules called coenzymes. Only when both the protein and its required cofactors are associated into the holoenzyme structure can the enzyme perform its biological role in accelerating chemical reactions.

**222.** (C) Metalloenzyme
A metalloenzyme is a type of enzyme that contains one or more tightly bound metal ions that are essential for its catalytic function and structural integrity. These ions are integrated into the protein framework during folding and remain associated with the enzyme throughout its life cycle. Examples include the zinc in carbonic anhydrase or the iron in cytochromes. This permanent association distinguishes metalloenzymes from metal-activated enzymes, which require loosely associated metal ions for activity.

**223.** (B) Cleavage by elimination leaving double bonds.
Lyases, classified under the fourth major enzyme class, specialize in catalyzing the cleavage of various chemical bonds by mechanisms other than hydrolysis or oxidation. These reactions often involve the elimination of a group, which leads to the formation of a double bond within the product. Alternatively, lyases can catalyze the addition of a group to a double bond. Decarboxylases and dehydratases are common examples of enzymes that perform these fundamental metabolic transformations.

**224.** (D) Maintain the 3D architecture of the active site.
Although the active site of an enzyme occupies only a small fraction of its total volume, the remaining protein structure is vital for biological function. This larger framework provides the necessary stability and architectural support to maintain the precise three-dimensional arrangement of the active site residues. By ensuring that catalytic groups are correctly positioned and oriented, the bulk of the protein allows the enzyme to achieve high specificity and efficient rate enhancement.

**225.** (A) P-1, Q-4, R-3, S-2
Kinetic parameters provide essential information about an enzyme's performance. Maximum velocity is the catalytic rate when the enzyme is fully saturated. The Michaelis constant is the substrate concentration at half $V_{\{}\max_{\}}$

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**242.** (C) Exponential signal amplification.
Protein cascades, such as those involved in blood clotting or hormonal responses, utilize a series of sequential enzyme activations to achieve exponential signal amplification. In these pathways, each active enzyme molecule can activate many molecules of a downstream target, which in turn activate even more downstream enzymes. This mechanism ensures that a very small initial stimulus can rapidly produce a massive and coordinated biological response, providing efficient regulation and control.

**243.** (A) Adapt reactions to tissue requirements.
The presence of multiple isoenzymes for proteins like lactate dehydrogenase is biologically important because it allows different organs to tailor metabolic reactions to their specific needs. Each isoenzyme possesses unique kinetic and regulatory properties that are optimized for the physiological conditions of the tissue where it is expressed. This metabolic flexibility ensures that pathways like glycolysis can function efficiently across various organs, from highly aerobic heart tissue to anaerobic skeletal muscle.

**244.** (D) RNA forms complex 3D structures for catalysis.
Ribozymes prove that catalytic power is not exclusively restricted to proteins. These specialized RNA molecules can fold into intricate three-dimensional structures that create specific active sites. By using these sites to stabilize transition states and orient substrates, ribozymes can accelerate biochemical reactions with an efficiency that rival proteins. This discovery has profound implications for our understanding of early life and the evolutionary transition from nucleic acid catalysts to protein-based systems.

**245.** (D) Prosthetic group.
A prosthetic group is a non-protein organic or inorganic molecule that is tightly and permanently bound to an enzyme. These groups, such as the heme ring found in cytochromes and hemoglobin, are essential for the protein's catalytic function or oxygen-binding ability. Unlike soluble coenzymes that dissociate after the reaction, prosthetic groups remain associated with the enzyme structure throughout its entire functional life, often providing critical redox or coordination centers.

**246.** (B) Tightly bound metal ions in the active site.
Metalloenzymes are distinguished by the presence of metal ions that are tightly and permanently integrated into the enzyme's active site and three-dimensional structure. These ions are essential for the enzyme's catalytic mechanism and remain bound during purification. This contrasts with metal-activated enzymes, which rely on loosely associated metal ions from the surrounding medium. In metalloenzymes, the metal often serves as a key participant in electron transfer or the polarization of substrates.

**247.** (D) Transferase.
Transferases are a major class of enzymes that catalyze the movement of a specific chemical functional group from one donor molecule to another acceptor molecule. This group can be a methyl, acyl, phosphate, or amino group. For example, kinases transfer phosphate groups from ATP to various proteins. These enzymes are essential for numerous biological processes, including signal transduction, energy metabolism, and the biosynthesis of complex cellular components like lipids and carbohydrates.

**248.** (A) Position and stabilize transition states.
The active site of an enzyme is a specialized microenvironment where specific amino acid side chains are precisely positioned to facilitate catalysis. These residues work together to recognize the substrate and, more importantly, to stabilize the high-energy transition state of the reaction. By providing a medium that lowers the activation energy and optimally orients the substrate's bonds, the active site enables the enzyme to achieve massive rate enhancements and high specificity.

**249.** (D) Samples multiple states prior to binding.
The conformational selection model proposes that enzymes are not static but exist in a dynamic equilibrium among multiple structural states. Rather than the substrate forcing a shape change, the substrate selectively binds to the pre-existing conformation that is most complementary to its structure. This binding then shifts the population of enzyme molecules toward that specific active state. This view emphasizes the inherent flexibility of proteins and their ability to sample different conformations.

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sites that perfectly complement these analogs. By exploiting this maximal binding energy, transition-state analogs bind with exceptionally high affinity, effectively locking the active site and preventing any further reaction from occurring.

**234.** (B) 10 degree Celsius increase.
The Q10 temperature coefficient is a measure of the sensitivity of a biological or chemical process to temperature changes. It specifically quantifies the factor by which the rate of an enzymatic reaction increases following a ten-degree Celsius rise in the surrounding temperature. Most enzymes exhibit a Q10 value of approximately two, indicating that the reaction rate roughly doubles for every ten-degree increase within the enzyme's stable temperature range.

**235.** (A) I, III and V
Discontinuous enzyme assays involve taking samples from a reaction at specific time intervals and immediately stopping the process using quenching agents like heat or acid. The amount of product formed at each time point is then measured using separate analytical techniques. This method is particularly useful for studying reactions that do not produce a convenient optical signal, allowing for the calculation of the initial velocity based on several discrete data points.

**236.** (C) Enzyme activity per unit of total protein.
Specific activity is a fundamental metric used to assess the purity of an enzyme preparation. It is defined as the total number of units of enzymatic activity divided by the total mass of all protein in the sample. As a purification protocol successfully removes contaminating non-catalytic proteins, the specific activity of the sample will increase. This value reaches its maximum only when the enzyme is isolated in its completely pure form.

**237.** (D) Sonication
Sonication is a physical disruption method that utilizes high-frequency sound waves to lyse cells. These waves generate acoustic cavitation, where microscopic bubbles form and rapidly collapse within the liquid medium. The violent collapse of these bubbles produces intense localized shear stress and pressure changes that tear open cell membranes and walls. This technique is effective for releasing intracellular enzymes, though care must be taken to prevent excessive heat generation during the process.

**238.** (C) The protein net charge is zero.
Isoelectric precipitation is a technique used in protein purification that relies on the fact that proteins exhibit their minimum solubility at their isoelectric point. This is the specific pH at which the protein molecule carries no net electrical charge. Without electrostatic repulsion between molecules, the proteins aggregate through hydrophobic interactions and precipitate from the solution. By adjusting the pH to the pI of a target protein, it can be selectively recovered.

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**239.** (B) Molecular size and hydrodynamic shape.
Gel filtration chromatography, also known as size-exclusion chromatography, separates protein molecules based on their molecular size and overall hydrodynamic shape. The stationary phase consists of porous beads that allow small proteins to enter while excluding larger ones. Consequently, large proteins travel around the beads and elute first, while smaller proteins must traverse the internal volume of the pores, following a longer path and eluting from the column at a later time.

**240.** (D) Isoelectric point and molecular mass.
Two-dimensional electrophoresis is a high-resolution separation technique that resolves complex mixtures of proteins. In the first dimension, proteins are separated by their isoelectric point using isoelectric focusing. This gel is then placed onto an SDS-PAGE gel, where the proteins are separated in the second dimension based on their molecular mass. This combination provides exceptional resolution, as proteins with the same pI are likely to have different masses, and vice-versa.

**241.** (A) Positive cooperative ligand binding.
The Hill coefficient is a mathematical parameter used to quantify the degree of cooperativity in an allosteric enzyme. A Hill coefficient greater than 1.0 indicates positive cooperativity, where the binding of a substrate molecule to one subunit increases the affinity for substrate at the remaining subunits. This behavior results in a sigmoidal kinetic profile, allowing the enzyme to respond with high sensitivity to small changes in substrate concentration within the cell.

in somatic cells.
Reason (R): DNA polymerase can only synthesize DNA in the 5' to 3' direction and requires an RNA primer, leading to the end-replication problem.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001

**21.** Which prokaryotic enzyme is responsible for the removal of RNA primers and their replacement with DNA?
(A) DNA Polymerase II
(B) DNA Polymerase I
(C) DNA Polymerase III
(D) Primase

2018

**22.** In eukaryotes, which DNA polymerase is primarily responsible for synthesizing the leading strand?
(A) DNA Polymerase $\alpha$
(B) DNA Polymerase $\delta$
(C) DNA Polymerase $\varepsilon$
(D) DNA Polymerase $\gamma$

2009

**23.** The termination of DNA replication in Escherichia coli involves the interaction of specific ter sites with which protein?
(A) DnaA
(B) DnaB
(C) SeqA
(D) Tus

2024

**24.** What is the primary role of the ORC (Origin Recognition Complex) in eukaryotes?
(A) Binding to the replication origin to initiate DNA synthesis
(B) Synthesizing the RNA primer
(C) Unwinding the double helix at the replication fork
(D) Ligating Okazaki fragments

2003

**25.** The process of DNA synthesis always proceeds in which direction?
(A) 3' to 5'
(B) 5' to 3'
(C) Both directions simultaneously on the same strand
(D) Depends on the specific DNA polymerase

2008

**26.** Which type of library explicitly contains only the expressed sequences of a specific tissue at a given time?
(A) Genomic library
(B) cDNA library
(C) BAC library
(D) YAC library

2012

**27.** When constructing a cDNA library, the first essential enzymatic step utilizes:
(A) Reverse transcriptase
(B) DNA ligase
(C) Restriction endonuclease
(D) Taq polymerase

2024

**28.** Functional complementation is a method utilized primarily for:
(A) Sequence determination of the target nucleotides
(B) Quantification of specific mRNA expression levels
(C) Gene isolation via mutant phenotype restoration
(D) Random mutation generation in a target sequence

2009

**29.** Which vector is most appropriate for constructing a genomic library of large eukaryotic DNA fragments ($> 100$ kb)?
(A) pBR322
(B) Phage lambda
(C) Expression plasmid
(D) BAC (Bacterial Artificial Chromosome)

2001

**30.** Read the following statements regarding genomic and cDNA libraries:
I. Genomic libraries contain introns and regulatory sequences.
II. cDNA libraries are uniform across all cell types of an organism.
III. cDNA libraries are generated using mRNA as a template.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

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**250.** (A) Free energy of activation ($\Delta G^{\dagger}$)
Enzymatic catalysis accelerates chemical reactions by specifically lowering the free energy of activation. This is the energy barrier that must be overcome for the ground-state reactants to reach the high-energy transition state. By providing a reaction pathway with more stable intermediates and optimized binding interactions, enzymes reduce this energy requirement. This allows a larger fraction of molecules to react per unit of time, significantly increasing the overall rate of the transformation.

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# CHAPTER 04
# Molecular Genetics

2003

**1.** Seymour Benzer proposed the classical term "cistron" to represent which of the following genetic units?
(A) The unit of genetic function
(B) The specific unit of genetic mutation
(C) The unit of genetic recombination events
(D) The unit of independent genetic assortment

2005

**2.** Which concept fundamentally challenged the classical "one gene-one polypeptide" hypothesis?
(A) The identification of single-point genetic mutations
(B) The bacterial process of generalized phage transduction
(C) Alternative splicing and overlapping genes
(D) The mechanism of semi-conservative DNA replication

2016

**3.** What is the primary functional difference between a muton and a recon?
(A) Mutation and recombination units, respectively.
(B) Functional and mutational genetic units, respectively.
(C) RNA-based and DNA-based genetic units, respectively.
(D) Cistronic and non-cistronic genetic units, respectively.

2024

**4.** Read the following statements regarding non-coding RNAs (ncRNAs):
I. They are transcribed from DNA but not translated into proteins.
II. They include tRNA and rRNA as structural components.
III. They lack any regulatory function in eukaryotes.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2012

**5.** The modern gene concept has evolved to include overlapping genes. Which organism's genome provided the first evidence of overlapping genes?
(A) Escherichia coli
(B) Bacteriophage $\phi$ X174
(C) Drosophila melanogaster
(D) Saccharomyces cerevisiae

2019

**6.** How many base pairs per turn are typically found in the standard Watson-Crick B-DNA double helix?
(A) 9.5
(B) 10.4
(C) 11.0
(D) 12.0

2001

**7.** Which helical form of DNA is favored under conditions of dehydration and is characterized by an 11-bp-per-turn right-handed structure?
(A) B-DNA
(B) Z-DNA
(C) H-DNA
(D) A-DNA

2009

**8.** What is the defining structural characteristic of Z-DNA compared to B-DNA?
(A) Left-handed helix with a zig-zag backbone
(B) Single-stranded sequence with no helical twist
(C) Triple-helical structure with Hoogsteen base pairs
(D) Right-handed helix with twelve base pairs per turn

2007

**9.** Identify the INCORRECT statement regarding the biochemical differences between prokaryotic and eukaryotic genomic DNA.
(A) Prokaryotic genomes are typically circular, whereas eukaryotic nuclear genomes are linear.
(B) Prokaryotic DNA is located in a membrane-bound nucleus.
(C) Eukaryotic DNA is heavily complexed with

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histones, unlike typical prokaryotic DNA.
(D) Eukaryotic genomes contain significantly more repetitive DNA sequences.

2010

**10.** Match the DNA conformation in List I with its corresponding attribute in List II.
List I: (DNA Conformation)
P. B-DNA
Q. A-DNA
R. Z-DNA
S. H-DNA
List II: (Attribute)
1. Triple-stranded structure
2. Physiological right-handed helix
3. Dehydrated right-handed helix
4. Left-handed helix
(A) P-2, Q-3, R-4, S-1
(B) P-3, Q-2, R-1, S-4
(C) P-2, Q-4, R-3, S-1
(D) P-1, Q-3, R-4, S-2

2008

**11.** In prokaryotic genome architecture, which of the following acts as a nucleoid-associated protein (NAP) facilitating DNA compaction?
(A) HU protein
(B) Histone H1
(C) Histone H3
(D) High Mobility Group (HMG) proteins

2010

**12.** Plasmids that cannot co-exist in the same bacterial cell without selection pressure are said to belong to the same:
(A) Conjugation group
(B) Recombination group
(C) Compatibility group
(D) Transduction unit

2013

**13.** Which enzyme primarily introduces negative supercoils into the circular DNA of Escherichia coli?
(A) DNA Ligase
(B) DNA Polymerase I
(C) Helicase
(D) DNA Gyrase

2019

**14.** What is the primary characteristic of a relaxed plasmid?
(A) Replication stringently coupled to host cell cycles
(B) Maintenance of a single plasmid copy per host cell
(C) Independent high copy number replication
(D) Total absence of any supercoiling within the DNA

2022

**15.** The basic structural unit of eukaryotic chromatin, consisting of DNA wrapped around an octamer of histones, is called a:
(A) Solenoid
(B) Nucleosome
(C) Chromatosome
(D) Replisome

2001

**16.** Which histone protein is not a component of the core octamer but binds to the linker DNA?
(A) H2A
(B) H2B
(C) H3
(D) H1

2013

**17.** The solenoid model of chromatin structure predicts a fiber with an approximate diameter of:
(A) 10 nm
(B) 30 nm
(C) 300 nm
(D) 700 nm

2021

**18.** Euchromatin is generally characterized by being:
(A) Highly condensed and transcriptionally inactive
(B) Devoid of histone proteins
(C) Less condensed and transcriptionally active
(D) Exclusively located at the centromere

2021

**19.** What is the specific function of telomeric repeats in eukaryotic chromosomes?
(A) Chromosome end protection from degradation
(B) Spindle fiber attachment during mitotic division
(C) Initiation sites for eukaryotic DNA replication
(D) Transcription of ribosomal RNA coding sequences

2017

**20.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Eukaryotic linear chromosomes undergo shortening with each replication cycle

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, reflecting affinity. The turnover number represents the molecules converted per active site per second. Finally, the ratio of the turnover number to the Michaelis constant serves as a measure of catalytic efficiency, indicating how effectively the enzyme operates at low substrate concentrations.

**226.** (C) A structural shift aligning catalytic groups.
According to the induced-fit model, enzymes are dynamic and flexible molecules. The initial binding of a substrate to the active site induces a significant conformational change, or structural shift, in the enzyme. This adjustment optimizes the orientation of catalytic functional groups relative to the substrate's bonds. This induced fit ensures that the enzyme achieves its most catalytically active state only when the correct substrate is present, maximizing the stabilization of the transition state.

**227.** (A) Molecules are at the peak of the energy barrier.
The transition state represents the highest energy point on the reaction coordinate between reactants and products. At this fleeting moment, chemical bonds are in the process of being formed and broken, and the molecules are at the peak of the activation energy barrier. This state is unstable and exists for only a fraction of a second. Enzymes function by stabilizing this high-energy intermediate, thereby reducing the energy required to reach it and accelerating the reaction.

**228.** (D) Irreversibly denaturing the protein backbone.
Metal ions in enzyme active sites contribute to catalysis through several productive mechanisms, including shielding negative charges, facilitating redox reactions, and polarizing water molecules to generate nucleophiles. They also help to orient substrates in the optimal position for the chemical reaction. However, these metal ions are essential for the enzyme's function and do not cause denaturation of the protein backbone; on the contrary, they often stabilize the enzyme's native three-dimensional structure.

**229.** (B) ES complex concentration remains constant.
The steady-state assumption is a fundamental principle used to simplify the mathematical derivation of the Michaelis-Menten equation. It posits that during the initial phase of the reaction, the concentration of the enzyme-substrate complex reaches a constant level. This implies that the rate at which the complex is formed from free enzyme and substrate is exactly balanced by the rate at which it breaks down into products or reverts back to its original components.

**230.** (C) $\frac{K_m}{V_{\max}}$
In a Lineweaver-Burk plot, the double reciprocal of the reaction velocity is graphed against the double reciprocal of the substrate concentration. This transformation produces a straight line where the slope is equal to the ratio of the Michaelis constant to the maximum velocity. This graphical method allows for the easy determination of these two key kinetic constants, providing important insights into the enzyme's substrate affinity and its overall catalytic capacity under specific conditions.

**231.** (D) First substrate must bind before second substrate binds.
An ordered sequential bi-bi mechanism is a type of multi-substrate reaction where the substrates must enter the enzyme's active site in a mandatory, specific sequence. The first substrate binds to the free enzyme, and only then can the second substrate bind to form a ternary complex. Similarly, the resulting products are released from the enzyme in a defined order. This mechanism is characteristic of many enzymes involved in energy metabolism and cellular signaling.

**232.** (A) Parallel lines with shifting intercepts.
Uncompetitive inhibition is uniquely identified in a Lineweaver-Burk plot by the appearance of parallel lines at different inhibitor concentrations. This occurs because the inhibitor binds only to the enzyme-substrate complex, reducing both the maximum velocity and the apparent Michaelis constant by exactly the same factor. Since the ratio of these two parameters remains constant, the slope of the line does not change, leading to parallel shifts in both the horizontal and vertical intercepts.

**233.** (D) Exploit binding energy intended for the transition state.
Transition-state analogs are extremely potent inhibitors because they are structurally designed to mimic the reaction's high-energy transition state. Since enzymes have evolved to bind this state much more tightly than the ground-state substrate to achieve catalysis, they possess active

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2023

**31.** Southern blotting is a hybridization-based technique utilized specifically for the detection of:
(A) Specific RNA sequences
(B) Specific DNA sequences
(C) Specific protein sequences
(D) Post-translational modifications

2016

**32.** Northern blotting primarily differs from Southern blotting in that Northern blotting is used to detect:
(A) Proteins instead of DNA
(B) DNA instead of RNA
(C) Lipids instead of nucleic acids
(D) RNA instead of DNA

2011

**33.** The initial denaturation step in a standard PCR cycle typically occurs at which temperature range?
(A) 50-60° C
(B) 70-75° C
(C) 94-98° C
(D) 100-110° C

2024

**34.** What is the primary role of a probe in hybridization-based gene identification methods?
(A) Cleaving target DNA at highly specific sequences
(B) Acting as primers for genomic DNA polymerases
(C) Binding to targets for complementary detection
(D) Degrading non-target nucleic acids in the sample

2024

**35.** PCR-based amplification relies heavily on which thermally stable enzyme?
(A) Taq DNA polymerase
(B) Reverse transcriptase
(C) Klenow fragment
(D) T4 DNA ligase

2008

**36.** Split genes consist of alternating sequences known as:
(A) Promoters and terminators
(B) Enhancers and silencers
(C) Leaders and trailers
(D) Exons and introns

2016

**37.** Which classification of introns undergoes self-splicing utilizing a guanosine nucleotide cofactor?
(A) Group II introns
(B) Group I introns
(C) Group III introns
(D) Spliceosomal introns

2017

**38.** Spliceosomal introns are typical of eukaryotic nuclear pre-mRNAs and are excised via a large ribonucleoprotein complex called the spliceosome. The core components of this complex are:
(A) rRNAs
(B) tRNAs
(C) snRNPs
(D) snoRNAs

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2004

**39.** During the splicing of spliceosomal introns, the intermediate structure formed through a 2'-5' phosphodiester bond is termed a:
(A) Hairpin loop
(B) Cruciform
(C) Holliday junction
(D) Lariat

2011

**40.** Read the following statements regarding intron classes:
I. Group II introns utilize an internal adenine branch point for splicing.
II. Group I introns require a spliceosome for excision.
III. Spliceosomal introns predominantly follow the GU-AG rule at their boundaries.
IV. Introns are never found in prokaryotic organisms.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and IV
(D) III and IV

2009

**41.** Overlapping genes are most frequently observed in which type of genomes to maximize coding efficiency?
(A) Mammalian genomes
(B) Viral genomes
(C) Plant genomes
(D) Fungal genomes

2025

**42.** Non-functional genomic sequences that resemble functional genes but contain premature stop codons or frameshifts are known as:
(A) Pseudogenes
(B) Oncogenes
(C) Transgenes
(D) Isoforms

2017

**43.** A processed pseudogene typically lacks which of the following structural elements?
(A) Introns and functional promoters
(B) The presence of a poly-A tail
(C) Protein-coding exonic segments
(D) Premature or internal stop codons

2003

**44.** The evolutionary significance of overlapping genes relates primarily to:
(A) Enhancing the frequency of recombination events
(B) Expanding the total size of the cellular genome
(C) Coding multiple sequences in compact spaces
(D) Providing resistance against chemical mutagens

2009

**45.** In bacteriophage $\phi$ X174, the phenomenon of overlapping genes was demonstrated by:
(A) Alternative splicing of primary transcripts
(B) Post-translational cleavage of polyproteins
(C) Independent transcription from distinct sites
(D) Use of different reading frames

2003

**46.** The TATA box in eukaryotic core promoters is generally located approximately how many base pairs upstream of the transcription start site?
(A) -10 bp
(B) -25 to -30 bp
(C) -75 to -80 bp
(D) -100 bp

2014

**47.** Which element is capable of elevating the transcription rate of a eukaryotic gene irrespective of its orientation or distance from the promoter?
(A) TATA box
(B) Enhancer
(C) Initiator element (Inr)
(D) Silencer

2003

**48.** Enhancers interact with the basal transcription machinery primarily through the formation of:
(A) Formation of large RNA lariat structures
(B) Stable triple-stranded DNA conformations
(C) Production of specific RNA-DNA hybrids
(D) DNA loops mediated by protein complexes

2005

**49.** Response elements in eukaryotic promoters allow coordinate regulation of multiple genes in response to:
(A) Specific physiological signals like hormones or heat shock
(B) Alternative splicing
(C) Random spontaneous mutations
(D) Telomere shortening

2002

**50.** Read the following statements regarding eukaryotic promoter elements:
I. Core promoters are strictly necessary for the assembly of the pre-initiation complex.
II. Silencers function by binding repressor proteins to decrease transcription.
III. Enhancers must be located within 50 base pairs of the start site.
IV. Response elements are only found downstream of the coding region.
V. The CAAT box regulates the frequency of transcription initiation.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and V
(C) II, III and V
(D) I, III and IV

2016

**51.** In the lac operon of Escherichia coli, the repressor protein is encoded by which gene?
(A) lacI
(B) lacZ
(C) lacY
(D) lacA

2002

**52.** The lac operon exhibits positive regulation dependent on glucose levels. Which complex binds to the promoter region to stimulate transcription when glucose is low?
(A) Lac repressor - allolactose complex
(B) RNA polymerase - sigma 70
(C) Trp repressor
(D) CRP (CAP) - cAMP complex

2015

**53.** The regulation of the trp operon involves both repression and a secondary mechanism known as attenuation. Attenuation relies directly on:

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(A) The intracellular concentration of cAMP
(B) The coupling of transcription and translation in prokaryotes
(C) The binding of the repressor to the operator
(D) Post-translational modification of the enzymes

2016

**54.** In the arabinose (ara) operon, the AraC protein acts as:
(A) Only an activator
(B) Only a repressor
(C) Both a positive and negative regulator depending on arabinose availability
(D) An RNA polymerase subunit

2015

**55.** The formation of the 3-4 terminator hairpin in the trp operon leader mRNA occurs when:
(A) Intracellular tryptophan levels are low
(B) Ribosome stalling at the tandem Trp codons
(C) RNA polymerase detaching from the template
(D) High tryptophan levels enable rapid translation of the leader peptide

2008

**56.** Which eukaryotic RNA polymerase is responsible for the synthesis of mRNA precursors?
(A) RNA Polymerase II
(B) RNA Polymerase I
(C) RNA Polymerase III
(D) RNA Polymerase IV

2008

**57.** The addition of a 7-methylguanosine cap to the 5' end of a nascent eukaryotic transcript occurs via an unusual:
(A) 3'-5' phosphodiester linkage
(B) 5'-5' triphosphate linkage
(C) 2'-5' phosphodiester linkage
(D) Peptide bond

2024

**58.** The 3' polyadenylation of eukaryotic mRNA requires a specific consensus sequence signal in the pre-mRNA, which is typically:
(A) Shine-Dalgarno sequence
(B) AAUAAA
(C) TATAAT
(D) GC box

2001

**59.** Which general transcription factor in eukaryotes is responsible for phosphorylating the C-terminal domain (CTD) of RNA Polymerase II to initiate elongation?
(A) TFIIH
(B) TFIID
(C) TFIIB
(D) TFIIA

2025

**60.** Match the Eukaryotic RNA Polymerase in List I with its primary transcript product in List II.
List I: (Polymerase)
P. RNA Polymerase I
Q. RNA Polymerase II
R. RNA Polymerase III
S. RNA Polymerase (Plant specific)
List II: (Transcript)
1. mRNA
2. rRNA (28S, 18S, 5.8S)
3. siRNA
4. tRNA and 5S rRNA
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-2, Q-4, R-1, S-3

2007

**61.** Spontaneous mutations resulting from the transient conversion of a nitrogenous base into an alternative isomer are called:
(A) Transversions
(B) Frameshifts
(C) Deletions
(D) Tautomeric shifts

2018

**62.** The spontaneous deamination of 5-methylcytosine in DNA yields:
(A) Uracil
(B) Adenine
(C) Thymine
(D) Guanine

2009

**63.** Replication slippage, which frequently occurs at short tandem repeats, primarily leads to which type of mutations?
(A) Large chromosomal translocations
(B) Base substitutions (transitions)
(C) Insertions or deletions (frameshifts)
(D) Nonsense mutations

2005

**64.** The loss of a purine base from the DNA backbone due to the cleavage of the glycosidic bond is known as:
(A) Deamination

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(B) Tautomerization
(C) Alkylation
(D) Depurination

2023

**65.** If a spontaneous mutation changes an adenine to a cytosine, this specific class of base substitution is a:
(A) Transversion
(B) Transition
(C) Silent mutation
(D) Frameshift

2015

**66.** Which class of chemical mutagens intercalates between adjacent DNA base pairs, causing frameshift mutations?
(A) Base analogs
(B) Alkylating agents
(C) Intercalating agents
(D) Deaminating agents

2002

**67.** Exposure to Ultraviolet (UV) light predominantly damages DNA by inducing the formation of:
(A) Pyrimidine dimers (e.g., thymine dimers)
(B) Double-strand breaks
(C) Alkylated bases
(D) DNA-protein crosslinks

2017

**68.** Ethidium bromide and acridine orange are classic examples of:
(A) Base analogs
(B) Alkylating agents
(C) Radiomimetic compounds
(D) Intercalating agents

2003

**69.** 5-bromouracil (5-BU) is a base analog that substitutes for thymine. In its enol form, it mispairs with which base, leading to transition mutations?
(A) Adenine
(B) Guanine
(C) Cytosine
(D) Uracil

2007

**70.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Ethyl methanesulfonate (EMS) is an effective mutagen commonly used in forward genetic screens.
Reason (R): EMS adds alkyl groups to nucleotide bases, frequently leading to $O^6$-ethylguanine formation and subsequent GC-to-AT transitions.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2016

**71.** Site-directed mutagenesis is an experimental technique used to:
(A) Random mutation generation across the genome
(B) Identifying rates of spontaneous mutations
(C) Extracting DNA from varied environmental sites
(D) Intentional changes in known DNA sequences

2003

**72.** In PCR-based site-directed mutagenesis utilizing overlap extension, the desired mutation is incorporated via the:
(A) Specially designed mutagenic primers
(B) Template DNA directly
(C) Restriction enzymes
(D) Taq DNA polymerase errors

2025

**73.** Cassette mutagenesis involves replacing a segment of target DNA with a synthetic DNA fragment (cassette). This process necessitates the presence of:
(A) Two identical restriction sites
(B) Unique flanking restriction endonuclease sites
(C) A highly processive RNA polymerase
(D) Viral integrases

2017

**74.** Oligonucleotide-directed mutagenesis was classically performed using the Kunkel method. This method strongly selects for the mutated DNA strand by utilizing a template containing:
(A) Uracil residues in place of thymine
(B) Methylated adenines
(C) Modified fluorescent bases
(D) 5-bromouracil

2019

**75.** A primary limitation of traditional PCR-based primer-extension mutagenesis is the risk of introducing:
(A) Risks of unintended polymerase error mutations
(B) Introduction of contaminating RNA

molecules
(C) Excessive supercoiling in the final products
(D) Automatic formation of premature stop codons

2005

**76.** Photoreactivation, a direct repair mechanism for UV-induced pyrimidine dimers, utilizes which enzyme?
(A) DNA ligase
(B) Endonuclease
(C) Photolyase
(D) Glycosylase

2017

**77.** Base excision repair (BER) relies initially on the action of which specific class of enzymes to remove the damaged base?
(A) AP endonucleases
(B) Exonucleases
(C) DNA helicases
(D) DNA glycosylases

2021

**78.** In E. coli, the mismatch repair (MMR) system distinguishes the newly synthesized DNA strand from the template strand because the template strand is:
(A) Acetylated
(B) Phosphorylated
(C) Methylated at specific sequence motifs
(D) Glycosylated

2018

**79.** The SOS response in E. coli is a global response to extensive DNA damage. It is negatively regulated by which repressor protein?
(A) RecA
(B) LexA
(C) UvrA
(D) DnaB

2017

**80.** Read the following statements regarding DNA repair pathways:
I. Non-homologous end joining (NHEJ) perfectly repairs double-strand breaks without sequence loss.
II. Nucleotide excision repair (NER) addresses bulky DNA lesions.
III. Homologous recombination (HR) provides an error-free mechanism for double-strand break repair.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) I only
(C) II and III
(D) III only

2013

**81.** Bacterial conjugation requires physical cell-to-cell contact, mediated primarily by the:
(A) Sex pilus
(B) Flagella
(C) Capsule
(D) Outer membrane porins

2011

**82.** In an F+ x F- mating, the typical outcome for the recipient cell is:
(A) It becomes an Hfr cell.
(B) It remains F- but acquires new chromosomal genes.
(C) It becomes F+.
(D) It dies due to genetic incompatibility.

2002

**83.** High frequency of recombination (Hfr) strains of E. coli are characterized by:
(A) The integration of the F plasmid into the bacterial chromosome
(B) The presence of multiple independent F plasmids
(C) An inability to perform conjugation
(D) The lack of a sex pilus

2023

**84.** When an F plasmid imprecisely excises from an Hfr chromosome, carrying adjacent bacterial genes with it, the resulting plasmid is designated as:
(A) F-
(B) R-plasmid
(C) Col-plasmid
(D) F'

2021

**85.** Interrupted mating experiments utilizing Hfr strains were historically significant for:
(A) Determining the molecular weight of the F plasmid
(B) Mapping the bacterial chromosome in minutes
(C) Isolating novel bacteriophages
(D) Studying the SOS response

2012

**86.** Bacterial transformation is defined as the:
(A) Transfer of DNA via a viral vector
(B) Transfer of DNA through a conjugation bridge

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(C) Uptake of naked exogenous DNA from the environment
(D) Mutation of DNA via physical mutagens

2001

**87.** A bacterial cell capable of taking up extracellular DNA is described as:
(A) Virulent
(B) Lysogenic
(C) Conjugative
(D) Competent

2004

**88.** Artificial competence is routinely induced in E. coli laboratory procedures utilizing:
(A) Calcium chloride treatment and heat shock
(B) Extreme ultraviolet irradiation
(C) Viral infection
(D) Ribonuclease treatment

2023

**89.** During natural transformation in Bacillus subtilis, the DNA enters the cell typically as:
(A) Intact double-stranded DNA
(B) A DNA-RNA hybrid
(C) A circular plasmid only
(D) Single-stranded DNA

2002

**90.** Read the following statements regarding homologous integration during transformation:
I. It requires sequence homology between the incoming DNA and the host chromosome.
II. It is mediated by the RecA protein in E. coli.
III. It results exclusively in plasmid formation.
IV. It only occurs in eukaryotes.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and IV
(D) III and IV

2006

**91.** The transfer of genetic material from one bacterium to another via a bacteriophage vector is termed:
(A) Transduction
(B) Transformation
(C) Conjugation
(D) Transfection

2014

**92.** Generalized transduction results from:
(A) The imprecise excision of a prophage sequence
(B) Integration of viral DNA into chromosomal loci
(C) Random packaging of host DNA into phage heads
(D) The specific uptake of plasmids by viral phages

2004

**93.** Specialized transduction differs from generalized transduction because it:
(A) Transfers any arbitrary segment of the host genome
(B) Requires cell-to-cell contact
(C) Utilizes virulent (lytic-only) phages
(D) Transfers only specific host genes flanking the prophage integration site

2001

**94.** Co-transduction frequency is utilized in bacterial genetics to determine:
(A) The exact base pair sequence of a gene
(B) The relative map distance between two closely linked genes
(C) The rate of spontaneous mutation
(D) The size of the bacteriophage capsid

2022

**95.** The classic example of a specialized transducing phage in E. coli is:
(A) T4 phage
(B) T7 phage
(C) Lambda phage
(D) $\phi$ X174

2021

**96.** Parasexual cycles in certain fungi achieve genetic recombination without:
(A) Plasmogamy
(B) Karyogamy
(C) Mitosis
(D) Meiosis and standard sexual structures

2015

**97.** The parasexual cycle was first discovered in which organism?
(A) Aspergillus nidulans
(B) Saccharomyces cerevisiae
(C) Neurospora crassa
(D) Penicillium notatum

2006

**98.** Mitotic recombination in heterozygous fungi occasionally produces patches of tissue that exhibit a recessive phenotype, a phenomenon observable via:
(A) Tetrad analysis
(B) Centromere mapping

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(C) Twin spots
(D) Cytoplasmic inheritance

2025

**99.** Tetrad analysis in Neurospora crassa is highly informative because its asci are:
(A) Linear, allowing the tracing of first and second division segregation
(B) Spherical, preventing analysis of meiotic divisions
(C) Diploid throughout their entire lifecycle
(D) Devoid of ascospores

2001

**100.** Read the following statements regarding tetrad analysis:
I. A Parental Ditype (PD) tetrad indicates unlinked genes if PD equals Non-Parental Ditype (NPD).
II. Tetratypes (TT) result from a crossover between one of the linked genes and its centromere.
III. Second Division Segregation (SDS) patterns are used for centromere mapping.
IV. Tetrad analysis is routinely performed in E. coli.
V. An excess of PD over NPD indicates genetic linkage.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II, III and V
(C) II, IV and V
(D) II, III and IV

2015

**101.** Recombination between two different viral genomes co-infecting the same host cell that involves segmented genomes is specifically referred to as:
(A) Genetic drift
(B) Genetic reassortment
(C) Transduction
(D) Splicing

2011

**102.** Which prominent human virus exploits genetic reassortment to produce pandemic strains?
(A) HIV
(B) Rabies virus
(C) Influenza A virus
(D) Herpes simplex virus

2018

**103.** In retroviruses, recombination frequently occurs during reverse transcription via a mechanism called:
(A) Reassortment
(B) Site-specific cleavage
(C) Splicing
(D) Copy-choice or template switching

2013

**104.** Seymour Benzer's fine-structure mapping utilized rapid-lysis (r) mutants of T4 phage. He mapped the rII locus based on the mutants' ability to form plaques on:
(A) E. coli B only
(B) E. coli K12(λ) only
(C) Both E. coli B and K12(λ)
(D) Neither strain

2005

**105.** In Benzer's rII mapping, a complementation test (cis-trans test) was performed. If two co-infecting rII mutants formed plaques on E. coli K12(λ), they were:
(A) In the same cistron
(B) In different cistrons
(C) Identical mutants
(D) Lacking the rII region entirely

2010

**106.** The simplest type of transposable element in prokaryotes, containing only the gene for transposase flanked by inverted repeats, is called an:
(A) Insertion Sequence (IS)
(B) R-plasmid
(C) Composite transposon
(D) Integron

2006

**107.** Composite transposons (such as Tn10) differ from insertion sequences primarily because they:
(A) Carry additional genes, such as antibiotic resistance genes, flanked by IS elements
(B) Lack inverted repeats
(C) Replicate independently in the cytoplasm
(D) Do not encode transposase

2022

**108.** In replicative transposition, the transposable element is duplicated, leaving one copy in the original site and placing a new copy at the target site. This process forms an intermediate structure called a:
(A) Lariat
(B) Holliday junction
(C) Cointegrate
(D) Replisome

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III. The DNA is wrapped completely three times around the core.

Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2001

**131.** Constitutive heterochromatin differs from facultative heterochromatin because constitutive heterochromatin:
(A) Is permanently condensed and predominantly localized at centromeres and telomeres
(B) Interconverts to euchromatin during the cell cycle
(C) Contains highly expressed housekeeping genes
(D) Is completely devoid of DNA methylation

2011

**132.** The eukaryotic centromere sequence primarily functions to:
(A) Direct the termination of replication
(B) Serve as an attachment point for the kinetochore complex during segregation
(C) Protect the chromosome from exonucleases
(D) Initiate RNA transcription

2021

**133.** Telomerase is biochemically unique because it functions as a:
(A) DNA-dependent RNA polymerase
(B) Restriction endonuclease
(C) Topoisomerase
(D) Reverse transcriptase carrying its own RNA template

2022

**134.** Which organism lacks a functional telomerase in most adult somatic cells, resulting in aging-related telomere shortening?
(A) Tetrahymena thermophila
(B) Saccharomyces cerevisiae
(C) Homo sapiens
(D) All prokaryotes

2005

**135.** The continuous DNA synthesis on the leading strand requires only a single RNA primer, whereas the lagging strand requires multiple primers to form:
(A) Klenow fragments
(B) Holiday junctions
(C) Replisomes
(D) Okazaki fragments

2011

**136.** In eukaryotic DNA replication, PCNA (Proliferating Cell Nuclear Antigen) functions identically to which prokaryotic replication component?
(A) β-clamp (sliding clamp)
(B) DnaG (Primase)
(C) Single-strand binding protein (SSB)
(D) DNA ligase

2009

**137.** Which enzyme seals the nicks in the sugar-phosphate backbone after RNA primer replacement?
(A) DNA polymerase I
(B) Topoisomerase II
(C) DNA ligase
(D) Helicase

2003

**138.** Eukaryotic chromosomes contain multiple origins of replication to overcome:
(A) The extreme length of linear chromosomes
(B) The lack of DNA polymerase
(C) The circular nature of the DNA
(D) The high mutation rate

NODIA

2022

**139.** In E. coli, DnaB protein acts functionally as a:
(A) Helicase
(B) Primase
(C) Clamp loader
(D) Ligase

2025

**140.** Read the following statements regarding DNA libraries:
I. A genomic library from liver cells differs extensively from a genomic library from brain cells of the same individual.
II. cDNA libraries utilize reverse transcriptase.
III. Functional complementation can screen libraries for specific phenotypes.
IV. Expression libraries require vectors with functional promoters.

Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) I only
(C) III and IV
(D) IV only

2012

**141.** Phage λ replacement vectors are advantageous for library construction because they:
(A) Exclusive replication via the lytic cycle

retrotransposition. A retrotransposed pseudogene is easily identified by the lack of:
(A) A poly-A track
(B) Mutations
(C) Stop codons
(D) Intron sequences

2008

**120.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Alternative splicing significantly increases the proteomic diversity of eukaryotic organisms without proportionally increasing genome size.
Reason (R): It allows multiple mRNA transcripts to be derived from a single primary transcript by selectively including or excluding exons.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**121.** The denaturation of double-stranded DNA into single strands exhibits a hyperchromic shift, which is characterized by:
(A) A decrease in UV absorbance at 260 nm
(B) An increase in UV absorbance at 260 nm
(C) An increase in absorbance at 280 nm
(D) The emission of fluorescence

2001

**122.** The major groove of B-DNA is functionally significant primarily because:
(A) It offers a sterically accessible, sequence-specific interaction site for regulatory proteins.
(B) It is the binding site for RNA polymerase strictly.
(C) It contains structural histones.
(D) It is narrower and deeper than the minor groove.

2011

**123.** The melting temperature (Tm) of a DNA double helix is directly proportional to the relative content of which base pair?
(A) C-G
(B) A-T
(C) A-U
(D) T-C

2019

**124.** Which form of DNA is an artifact of laboratory crystallization under high salt concentrations and is rarely physiological?
(A) B-DNA
(B) C-DNA
(C) A-DNA
(D) Z-DNA

2021

**125.** In the context of bacterial genome architecture, supercoiling is vital for genome compaction. Most physiological DNA exists in a state of:
(A) Positive supercoiling
(B) Complete relaxation
(C) Catenation
(D) Negative supercoiling

2022

**126.** A plasmid that transfers itself via conjugation must harbor which specific genetic region?
(A) oriC
(B) lacZ
(C) tra operon
(D) ter site

2004

**127.** Col plasmids in bacteria encode for:
(A) Resistance against heavy metals
(B) Proteins that destroy closely related bacterial strains
(C) Enzymes degrading rare carbohydrates
(D) Sex pili

2013

**128.** Replication of the E. coli chromosome begins precisely at:
(A) The ter region
(B) The promoter
(C) The Shine-Dalgarno sequence
(D) The oriC locus

2016

**129.** The partitioning of newly replicated bacterial chromosomes into daughter cells is facilitated by:
(A) The par system and interactions with the cell membrane/septum
(B) The spindle apparatus
(C) Actin-myosin interaction
(D) Centromeres and kinetochores

2019

**130.** Read the following statements detailing the eukaryotic nucleosome:
I. It contains approximately 147 base pairs of DNA.
II. The core octamer lacks histone H1.

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2011

**152.** Alternative splicing allows multiple protein isoforms to be produced from a single gene. A classic example in Drosophila sex determination involves the gene:
(A) Sxl (Sex-lethal)
(B) lacZ
(C) GAL4
(D) p53

2021

**153.** Group I introns undergo self-splicing using an exogenous cofactor. The nucleophile for the first transesterification reaction is:
(A) The 2'-OH of an internal branch-point Adenine
(B) The 3'-OH of a free Guanosine nucleotide
(C) The 5'-phosphate of a Cytosine
(D) A catalytic water molecule

2004

**154.** Trans-splicing, where exons from two separate RNA transcripts are ligated together, is characteristically observed in:
(A) Caenorhabditis elegans
(B) Escherichia coli
(C) Homo sapiens (predominantly)
(D) Saccharomyces cerevisiae

2003

**155.** Overlapping genes can be transcribed from:
(A) Opposite strands of the DNA in the same locus
(B) Only the sense strand of the DNA
(C) Only the antisense strand
(D) Distant chromosomal locations

2011

**156.** The overlapping genetic organization of the Hepatitis B virus (HBV) genome effectively achieves:
(A) Higher rates of spontaneous mutations
(B) Avoiding detection by host immune cells
(C) Multi-protein encoding within 3.2 kb
(D) Inhibition of the RNA splicing process

2004

**157.** Processed pseudogenes differ structurally from non-processed pseudogenes because processed pseudogenes arise from:
(A) Unequal crossing over
(B) Gene duplication events
(C) Viral transduction
(D) Retrotransposition of mature mRNA

2004

**158.** Ribosomal RNA (rRNA) genes in eukaryotes are transcribed by RNA Polymerase I primarily within the:
(A) Cytoplasm
(B) Endoplasmic reticulum
(C) Nucleoplasm
(D) Nucleolus

2009

**159.** The GC box, typically found upstream of eukaryotic promoters, is recognized and bound by which specific transcription factor?
(A) TBP (TATA-binding protein)
(B) Sp1
(C) Oct-1
(D) NF-κB

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2022

**160.** Match the regulatory element in List I to its specific functional consequence in List II.
List I: (Element)
P. Enhancer
Q. Silencer
R. TATA Box
S. Insulator
List II: (Consequence)
1. Blocks the interaction between an enhancer and a non-target promoter
2. Mediates basal transcription complex assembly
3. Binds regressors to downregulate transcription
4. Binds activators to upregulate transcription
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2005

**161.** Enhancers differ fundamentally from promoters in that enhancers:
(A) Can function effectively in either orientation
(B) Strictly require an exact distance from the start site
(C) Must be upstream of the gene
(D) Are transcribed into enhancer-RNAs only in prokaryotes

2024

**162.** The assembly of the eukaryotic pre-initiation complex begins with the binding of TFIID to the promoter. Which subunit of TFIID directly recognizes the TATA box?
(A) TFIIA
(B) TFIIB

(B) Host cell death occurring immediately on entry
(C) Maintenance of a single-stranded DNA genome
(D) Stuffer substitution with 10-20 kb inserts

2023

**142.** Colony hybridization involves transferring bacterial colonies to a membrane and screening them using a:
(A) Labeled antibody
(B) Specific restriction enzyme
(C) Radiolabeled or fluorescently labeled DNA/RNA probe
(D) Functional bioassay

2004

**143.** If an investigator wishes to isolate the gene encoding human insulin from a genomic library, utilizing a complementary mouse insulin gene sequence is an example of screening by:
(A) Functional complementation
(B) Immunological screening
(C) Heterologous probing
(D) Blue-white selection

2011

**144.** Chromosome walking is a technique primarily used for:
(A) Visualizing chromosomes during the metaphase
(B) Separating fragments by size via electrophoresis
(C) Degrading repetitive genomic DNA sequences
(D) Isolating overlapping fragments to map regions

2007

**145.** Southern blotting specifically utilizes which matrix for the size-based separation of restriction fragments before transfer?
(A) Agarose gel
(B) Polyacrylamide gel
(C) Silica column
(D) Nitrocellulose directly

2008

**146.** A critical step in Southern blotting prior to transfer is alkaline treatment of the gel. This treatment ensures:
(A) The degradation of RNA
(B) The activation of restriction enzymes
(C) The fixation of DNA to the gel matrix
(D) The denaturation of double-stranded DNA to allow probe hybridization

2011

**147.** Reverse Transcription PCR (RT-PCR) is specifically tailored for the amplification of:
(A) Expressed mRNA sequences translated into cDNA
(B) Methylated DNA promoters
(C) Telomeric repeat arrays
(D) Circular plasmids

2005

**148.** Real-Time Quantitative PCR (qPCR) differs from standard PCR primarily by:
(A) Using RNA polymerase instead of DNA polymerase
(B) Amplifying whole genomes simultaneously
(C) Avoiding the use of primers
(D) Measuring the amplification of DNA continuously during the exponential phase

2018

**149.** Microarrays fundamentally expand upon the principle of which hybridization technique by utilizing thousands of immobilized probes?
(A) Western blotting
(B) Northern blotting
(C) Southern blotting
(D) Eastern blotting

2006

**150.** Read the following statements concerning split genes and splicing:
I. Exons are the coding segments retained in mature mRNA.
II. Spliceosomes consist of snRNAs and proteins.
III. Group I and II introns require ATP and spliceosomes for excision.
IV. Intronic sequences are always degraded instantly and never have biological function.
V. The branch point sequence is essential for lariat formation in spliceosomal introns.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and V
(C) II, III and V
(D) I, III and IV

2016

**151.** Which invariant dinucleotide sequences typically define the 5' and 3' splice sites of spliceosomal introns, respectively?
(A) AG and GU
(B) AA and UU
(C) GC and AG
(D) GU and AG

NODIA

2024

**109.** Conservative (cut-and-paste) transposition lacks the cointegrate intermediate and relies on the element directly physically excising. This mechanism is characteristic of:
(A) Tn3
(B) Mu phage
(C) Retroviruses
(D) Tn10

2021

**110.** Match the transposable element feature in List I with its description in List II.
List I: (Feature)
P. Inverted Repeats (IR)
Q. Transposase
R. Resolvase
S. Direct Repeats (DR)
List II: (Description)
1. Enzyme required for cointegrate resolution
2. Host DNA sequence duplicated upon insertion
3. Element sequence recognized by excision enzymes
4. Enzyme responsible for DNA excision and integration
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2012

**111.** Class I transposable elements in eukaryotes replicate through an RNA intermediate. They are collectively known as:
(A) DNA transposons
(B) Insertion sequences
(C) Retrotransposons
(D) Plasmids

2004

**112.** The autonomous Ac (Activator) and non-autonomous Ds (Dissociation) transposable elements were discovered in which organism?
(A) Drosophila melanogaster
(B) Arabidopsis thaliana
(C) Saccharomyces cerevisiae
(D) Zea mays (Maize)

2008

**113.** Why is the Ds element in maize considered non-autonomous?
(A) Transposase mutation requiring Ac assistance
(B) Absence of functional flanking inverted repeats
(C) Mobility restricted to sexual reproduction cycles
(D) Requirement for RNA intermediates to transpose

2006

**114.** Long Interspersed Nuclear Elements (LINEs) in the human genome belong to which class of elements?
(A) Class II DNA transposons
(B) LTR retrotransposons
(C) Non-LTR retrotransposons
(D) Miniature Inverted-repeat Transposable Elements (MITEs)

2005

**115.** P elements in Drosophila melanogaster cause hybrid dysgenesis when:
(A) A female lacking P elements mates with a male carrying P elements.
(B) Both parents carry P elements.
(C) A female with P elements mates with a male lacking P elements.
(D) The elements are transcribed into siRNA.

2023

**116.** The concept of the gene as purely a functional unit (cistron) has been modified to account for phenomena where a single pre-mRNA can yield multiple distinct functional proteins. This process is:
(A) Overlapping genes
(B) Template switching
(C) Attenuation
(D) Alternative splicing

2001

**117.** Which of the following non-coding RNAs is fundamentally involved in RNA interference (RNAi)?
(A) snoRNA
(B) siRNA
(C) snRNA
(D) tRNA

2017

**118.** The "one gene-one enzyme" hypothesis was established using which experimental organism?
(A) Escherichia coli
(B) Mus musculus
(C) Caenorhabditis elegans
(D) Neurospora crassa

2011

**119.** Pseudogenes often arise structurally via two mechanisms: genomic duplication or

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(C) TBP (TATA-binding protein)
(D) TAFs

2008

**163.** Glucocorticoid Response Elements (GREs) are specific promoter sequences that bind:
(A) Zinc-finger containing steroid hormone receptors
(B) Non-specific RNA polymerase II
(C) The basic leucine zipper of AP-1
(D) Small nuclear RNAs

2018

**164.** The lac operon represents an inducible system. Its structural genes are significantly transcribed only when:
(A) Glucose is high and lactose is absent
(B) Glucose is high and lactose is present
(C) Glucose is low and lactose is absent
(D) Glucose is low and lactose is present

2025

**165.** The inducer of the lac operon, which directly binds and inactivates the lac repressor, is:
(A) Galactose
(B) Allolactose
(C) Lactose
(D) cAMP

2003

**166.** In the trp operon, the Trp repressor requires a co-repressor to bind the operator. This co-repressor is:
(A) Indole
(B) Shikimic acid
(C) Tryptophan
(D) cAMP

2008

**167.** Attenuation of the trp operon relies on a leader peptide containing tandem codons for:
(A) Methionine
(B) Tryptophan
(C) Phenylalanine
(D) Histidine

2012

**168.** In the absence of arabinose, the AraC protein represses the ara operon by binding to which two sites to form a DNA loop?
(A) araI1 and araO2
(B) araI1 and araI2
(C) araO1 and ara O2
(D) lacO and araI

NODIA

2023

**169.** During eukaryotic transcription termination, the RNA transcript is cleaved downstream of the polyadenylation signal. Which enzyme then adds the poly-A tail?
(A) RNA Polymerase II
(B) Ribonuclease
(C) DNA ligase
(D) Poly(A) polymerase (PAP)

2017

**170.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): The 5' cap of eukaryotic mRNA is crucial for translation initiation.
Reason (R): The 5' cap is recognized by translation initiation factors (e.g., eIF4E) and protects the mRNA from 5' to 3' exonucleases.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**171.** The spontaneous depurination of DNA leaves an abasic site. If unrepaired before replication, DNA polymerase typically inserts which base opposite the blank site?
(A) Adenine
(B) Cytosine
(C) Guanine
(D) Thymine

2009

**172.** Tautomeric shifts cause anomalous base pairing. The rare imino form of Adenine incorrectly pairs with:
(A) Thymine
(B) Guanine
(C) Cytosine
(D) Uracil

2006

**173.** Trinucleotide repeat expansion disorders, such as Huntington's disease, are primarily caused by which spontaneous mutation mechanism?
(A) Transposon insertion
(B) Deamination
(C) Unequal crossing over
(D) Replication slippage

complexed with histones, whereas prokaryotic DNA is typically circular and associated with different types of compaction proteins.

**10.** (A) P-2, Q-3, R-4, S-1
B-DNA is the standard right-handed helix found under physiological conditions. A-DNA forms during dehydration and is also right-handed but more compact. Z-DNA is a left-handed helix with a distinctive zig-zag backbone. H-DNA refers to a triple-stranded structure formed by Hoogsteen base pairing. These conformations illustrate the structural flexibility of DNA in response to different sequences and environmental conditions.

**11.** (A) HU protein
Bacteria utilize nucleoid-associated proteins to organize and compact their circular genomes. The HU protein is a prominent example in organisms like Escherichia coli. It binds to DNA non-specifically, inducing bends and facilitating the formation of supercoiled domains. This function is analogous to eukaryotic histones, though HU proteins lack the complex octameric structure and are much smaller in molecular weight.

**12.** (C) Compatibility group
Plasmids that share similar replication or partitioning mechanisms cannot be maintained stably within the same bacterial cell. This phenomenon is known as plasmid incompatibility. Plasmids that compete for the same cellular resources or regulatory factors belong to the same compatibility group. Without selective pressure, one plasmid will eventually be lost during cell division, ensuring only one type persists in the lineage.

**13.** (D) DNA Gyrase
DNA gyrase is a specialized type II topoisomerase essential for maintaining the topological state of the circular bacterial chromosome. It is uniquely capable of introducing negative supercoils into the DNA molecule using energy derived from ATP hydrolysis. This process relieves the positive supercoiling that accumulates ahead of the replication fork and ensures that the DNA remains accessible for transcription and replication.

**14.** (C) Independent high copy number replication
Plasmids are classified based on their copy number regulation within the host cell. A relaxed plasmid is one that replicates independently of the host's chromosomal replication cycle. This independence results in the maintenance of a high copy number, often reaching dozens or hundreds per cell. Such plasmids are valuable tools in molecular cloning due to the high yields of DNA they provide.

**15.** (B) Nucleosome
The nucleosome is the fundamental repeating unit of eukaryotic chromatin. It consists of a core of eight histone proteins around which approximately 147 base pairs of DNA are wrapped. This structure facilitates the first level of DNA compaction within the nucleus. The interaction between the negatively charged DNA backbone and the positively charged histone tails is critical for maintaining this structural integrity.

**16.** (D) H1
Eukaryotic chromatin organization involves several histone proteins. While H2A, H2B, H3, and H4 form the core octamer of the nucleosome, histone H1 functions as a linker histone. It binds to the site where DNA enters and exits the nucleosome core, as well as to the linker DNA. This binding helps stabilize the 30 nm fiber and promotes higher-order chromatin folding.

**17.** (B) 30 nm
The solenoid model describes the second level of chromatin organization beyond the beads-on-a-string nucleosomal arrangement. In this structure, the nucleosome chain coils into a helical fiber. This fiber has a characteristic diameter of approximately 30 nm. The formation of this structure is heavily dependent on the presence of linker histone H1 and is essential for the further compaction of genomic DNA.

**18.** (C) Less condensed and transcriptionally active
Eukaryotic chromatin is divided into two functional types: euchromatin and heterochromatin. Euchromatin is characterized by a less condensed structure, which allows transcription factors and RNA polymerase easy access to the DNA sequence. Consequently, these regions are transcriptionally active and contain most of the organism's protein-coding genes. In contrast, heterochromatin remains highly condensed and is typically associated with silenced genetic loci.

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(D) A bacteriophage

2023

**186.** The F-plasmid carries tra genes which are crucial for:
(A) Encoding the pilin protein and assembly machinery for the sex pilus
(B) Transcribing repressor proteins
(C) Mediating DNA integration into the host chromosome
(D) Resisting heavy metal toxicity

2002

**187.** The time mapping of E. coli via Hfr interrupted mating revealed that the bacterial chromosome is structurally:
(A) A single circular map
(B) A single linear molecule
(C) Multiple linear chromosomes
(D) Composed of fragmented linear segments

2005

**188.** Natural competence for transformation in Streptococcus pneumoniae requires the synthesis and extracellular accumulation of a signaling molecule known as:
(A) cAMP
(B) Competence stimulating peptide (CSP)
(C) Allolactose
(D) Inducer factor

2004

**189.** During specialized transduction by lambda phage, imprecise excision usually results in the viral incorporation of the closely linked bacterial gene:
(A) lac
(B) trp
(C) his
(D) gal

2021

**190.** Arrange the sequence of events during specialized transduction:
I. Prophage induction and imprecise excision.
II. Phage infects a new bacterial host.
III. Viral DNA integrates into the host chromosome, bringing the bacterial gene.
IV. Lytic cycle completes, releasing defective transducing phages.
V. Lysogenization of the original bacterial host.
(A) V → I → IV → II → III
(B) I → V → II → III → IV
(C) II → III → V → I → IV
(D) V → II → I → IV → III

2004

**191.** The primary distinction between generalized and specialized transduction is that generalized transduction can transfer:
(A) Only specific plasmid sequences
(B) Any gene in the bacterial chromosome
(C) Only genes adjacent to the attB integration site
(D) RNA exclusively

2016

**192.** Plaque morphology is a key phenotypic marker for bacteriophages. Rapid-lysis (r) mutants of T4 phage form:
(A) Unusually small, turbid plaques
(B) No plaques at all
(C) Pigmented plaques
(D) Unusually large, clear plaques

2015

**193.** Benzer defined the recon as the smallest unit of recombination. Through fine-structure mapping, he determined that a recon is equivalent physically to:
(A) A single nucleotide pair
(B) An entire gene
(C) An exon
(D) An operon

NODIA

2012

**194.** Viral recombination involving reassortment of segmented genomes represents a major mechanism for antigenic:
(A) Drift
(B) Shift
(C) Exclusion
(D) Mimicry

2005

**195.** Copy-choice recombination in viruses like HIV is facilitated by the low processivity and template-switching ability of:
(A) Reverse Transcriptase
(B) RNA Polymerase
(C) DNA Integrase
(D) Protease

2009

**196.** Fungal parasexual cycles are particularly important for generating genetic diversity in:
(A) Species that are exclusively sexual
(B) Prokaryotic organisms
(C) Pathogenic viruses
(D) Species lacking a known sexual cycle (Deuteromycetes)

2002

**220.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Eukaryotic genomes utilize multiple origins of replication simultaneously during the S-phase.
Reason (R): Eukaryotic DNA polymerases synthesize DNA significantly faster than prokaryotic polymerases, requiring fewer origins.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**221.** The replisome coordinates leading and lagging strand synthesis. In E. coli, this coordination is achieved by:
(A) Use of two distinct DNA Polymerase molecules
(B) Synthesis of the lagging strand in 3' to 5'
(C) Bypassing RNA primer needs on lagging strands
(D) Lagging strand looping to align polymerases

2018

**222.** Which technique utilizes a viral promoter (e.g., T7) inserted into a vector to generate functional protein products in vivo or in vitro?
(A) Genomic library construction
(B) Expression library screening
(C) Southern blotting
(D) Cassette mutagenesis

2023

**223.** To isolate a specific gene causing an observable mutant phenotype, researchers often construct a genomic library from the wild-type organism and introduce it into the mutant. This method is:
(A) Primer extension
(B) Genomic walking
(C) Functional complementation
(D) Northern blotting

2014

**224.** RFLP (Restriction Fragment Length Polymorphism) analysis combines which two molecular techniques?
(A) PCR and Northern blotting
(B) Mutagenesis and expression screening
(C) Microarray and RT-PCR
(D) Restriction enzyme digestion and Southern blotting

2007

**225.** Introns of the Group II class share a striking mechanistic and structural similarity to:
(A) Telomerase
(B) Spliceosomal introns
(C) Group I introns
(D) DNA transposons

2013

**226.** Alternative splicing of pre-mRNA is primarily regulated by specific RNA-binding proteins that bind to:
(A) Promoter elements
(B) The poly-A tail strictly
(C) Splicing enhancers and silencers (exonic or intronic)
(D) The 5' cap

2007

**227.** A functional pseudogene that has regained biological significance, usually by producing a regulatory lncRNA, is classified as a:
(A) Expressed or "alive" pseudogene
(B) Transgene
(C) Unitary pseudogene
(D) Processed pseudogene

2024

**228.** Eukaryotic transcription requires chromatin remodeling complexes to displace nucleosomes. These complexes utilize energy from:
(A) GTP hydrolysis
(B) NADH oxidation
(C) Proton motive force
(D) ATP hydrolysis

2001

**229.** Upstream Activating Sequences (UAS) in yeast fundamentally serve the same regulatory function as which element in higher eukaryotes?
(A) Core promoters
(B) Enhancers
(C) Silencers
(D) Terminators

2016

**230.** Select the correct sequence of events in Eukaryotic mRNA processing:
(A) Splicing → 5' Capping → 3' Polyadenylation
(B) 5' Capping → Splicing → 3' Polyadenylation
(C) 3' Polyadenylation → 5' Capping → Splicing
(D) 5' Capping → 3' Polyadenylation → Splicing

2021

**231.** The lac repressor regulates the lac operon. Structurally, the fully functional lac repressor protein exists as a:

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enzymatic activity is strictly responsible for both excision and integration?
(A) Transposase
(B) Reverse transcriptase
(C) Integrase
(D) Endonuclease

2005

**210.** Match the mutation type in List I with its resulting effect on the polypeptide in List II.
List I: (Mutation)
P. Silent mutation
Q. Missense mutation
R. Nonsense mutation
S. Frameshift mutation
List II: (Effect)
1. Introduces a premature stop codon
2. Alters a single amino acid
3. Alters the entire downstream amino acid sequence
4. Does not change the amino acid
(A) P-4, Q-2, R-1, S-3
(B) P-1, Q-3, R-2, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-2, Q-4, R-1, S-3

2001

**211.** Which conceptual model fundamentally replaced the strict "cistron" as alternative splicing was discovered?
(A) One gene-multiple transcripts/isoforms
(B) One gene-one polypeptide
(C) The central dogma strictly unidirectional
(D) The replicon model

2015

**212.** Overlapping genes can be identified computationally by detecting multiple reading frames that encode functional domains within the same:
(A) Protein sequence
(B) Intronic sequence
(C) UTR region
(D) Nucleotide sequence

2023

**213.** Non-coding RNAs (ncRNAs) such as microRNAs (miRNAs) execute significant regulatory functions by:
(A) Acting as templates for unique proteins
(B) Inducing targeted mRNA degradation or translational repression
(C) Integrating directly into chromosomal DNA
(D) Enhancing spontaneous mutation rates

NODIA

2012

**214.** In the topological description of DNA, the equation $Lk = Tw + Wr$ is used. Which term refers specifically to the number of times the double helix crosses over itself in 3D space?
(A) Twist (Tw)
(B) Writhe (Wr)
(C) Linking Number (Lk)
(D) Density

2004

**215.** Biochemical analysis of eukaryotic genomic DNA reveals a high proportion of repetitive sequences. The fastest renaturing fraction in Cot curve analysis represents:
(A) Single-copy genes
(B) Moderately repetitive genes
(C) Pseudogenes
(D) Highly repetitive (satellite) DNA

2004

**216.** Bacterial nucleoid domains are supercoiled independently, which restricts topological relaxation to a single loop if a nick occurs. This domain structure is maintained by:
(A) Nucleoid-associated proteins (NAPs) and RNA/protein tethering
(B) Histone H1
(C) Telomerases
(D) A rigid nuclear envelope

2011

**217.** Plasmids are utilized extensively as cloning vectors. The ColE1 plasmid regulates its replication and copy number using:
(A) A protein repressor
(B) Antisense RNA (RNA I)
(C) Stringent response alarmones
(D) Histone modifications

2014

**218.** Centromeric DNA in higher eukaryotes is typically characterized by:
(A) Highly transcribed house-keeping genes
(B) Large arrays of tandemly repeated satellite DNA
(C) Exclusively unique, single-copy sequences
(D) Absence of nucleosomes

2009

**219.** Telomeric DNA sequences in vertebrates typically consist of the tandem repeat:
(A) TTAGGG
(B) CCCTAA
(C) TTGGCC
(D) AATCCC

2017

**197.** Mitotic crossing over in the parasexual cycle is characterized by its frequency being:
(A) Equal to meiotic crossing over
(B) Much lower than meiotic crossing over
(C) Much higher than meiotic crossing over
(D) Dependent on the presence of bacteriophages

2019

**198.** The generation of haploid segregants from a diploid in the fungal parasexual cycle occurs via:
(A) Standard meiosis
(B) Progressive, random chromosome loss (haploidization)
(C) Endoreduplication
(D) Budding

2002

**199.** First division segregation (FDS) patterns in a linear fungal ascus indicate that:
(A) Crossing over occurred between the gene and centromere
(B) Gene localization directly at the centromere
(C) Multiple crossover events within the gene locus
(D) Absence of crossing over at the centromere

2023

**200.** The classic 4:4 segregation pattern in an 8-spore ascus of Neurospora is diagnostic for:
(A) First division segregation
(B) Second division segregation
(C) Gene conversion
(D) Nondisjunction

2025

**201.** Gene conversion, observed occasionally during tetrad analysis (e.g., 6:2 or 5:3 ratios), strongly supports the molecular model of recombination known as the:
(A) Conservative replication model
(B) Holliday model and mismatch repair of heteroduplex DNA
(C) Rolling circle model
(D) Replicon model

2014

**202.** Transposable elements are often described colloquially as:
(A) Pseudogenes
(B) Jumping genes
(C) Silent genes
(D) Ribozymes

2019

**203.** The inverted repeats (IRs) flanking an Insertion Sequence (IS) are crucial because they serve as:
(A) Recognition and binding sites for the transposase enzyme
(B) Promoters for transposase
(C) Ribosome binding sites
(D) Origins of replication

2009

**204.** Target site duplication (Direct Repeats) occurs during transposition because the transposase:
(A) Specifically synthesizes direct repeats de novo
(B) Makes staggered cuts in the target DNA, which are subsequently filled in by host DNA polymerase
(C) Replicates the inverted repeats twice
(D) Integrates a circular DNA intermediate

2003

**205.** The Ac (Activator) and Ds (Dissociation) transposable element system in maize visibly affects the phenotype of:
(A) Root nodule formation
(B) Leaf structure solely
(C) Stem elongation
(D) Kernel pigmentation

2009

**206.** Retrotransposons fundamentally differ from DNA transposons by utilizing an intermediate composed of:
(A) Single-stranded DNA
(B) RNA
(C) Protein
(D) Double-stranded circular DNA exclusively

2011

**207.** Alu elements, the most abundant transposable elements in the human genome, are classified as:
(A) LTR retrotransposons
(B) SINEs (Short Interspersed Nuclear Elements)
(C) LINEs (Long Interspersed Nuclear Elements)
(D) Class II DNA transposons

2021

**208.** The transposition of P elements in Drosophila is repressed in the somatic tissues of wild-type flies by:
(A) Heavy DNA methylation
(B) Complete degradation of the P elements
(C) Absence of RNA polymerase
(D) Specific repressor proteins synthesized from alternatively spliced transposase transcripts

2018

**209.** Class II DNA transposons excise and integrate via a "cut-and-paste" mechanism. Which

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(A) Monomer
(B) Dimer
(C) Hexamer
(D) Tetramer

2012

**232.** Catabolite repression of the lac operon ensures that the bacteria prefer glucose. When glucose is depleted, the level of which secondary messenger rises?
(A) cAMP
(B) cGMP
(C) IP3
(D) Calcium ions

2015

**233.** A nonsense mutation spontaneously converting a tyrosine codon (UAC) to a stop codon (UAA) is an example of a:
(A) Transition
(B) Transversion
(C) Frameshift
(D) Silent mutation

2012

**234.** Nitrous acid ($HNO_2$) acts as a potent mutagen by causing the oxidative deamination of specific bases. Deamination of cytosine produces:
(A) Uracil
(B) Adenine
(C) Guanine
(D) Thymine

2025

**235.** The error rate of Taq DNA polymerase is relatively high because it intrinsically lacks:
(A) 3' to 5' exonuclease (proofreading) activity
(B) 5' to 3' polymerase activity
(C) Processivity
(D) Heat stability

2012

**236.** To create a precise single amino acid substitution via PCR-based site-directed mutagenesis, the primer must contain the mismatch:
(A) Exactly at the 3' end
(B) Exactly at the 5' end
(C) Centrally located within the primer sequence
(D) Outside the complementary region

2021

**237.** Mutations in human mismatch repair (MMR) genes prominently lead to genetic instability and are the primary cause of:
(A) Xeroderma pigmentosum
(B) Sickle cell anemia
(C) Huntington's disease
(D) Hereditary Non-Polyposis Colorectal Cancer (HNPCC)

2017

**238.** Excision repair of bulky lesions (NER) in humans utilizes a complex of proteins, and genetic defects in these proteins typically result in the disease:
(A) Cystic fibrosis
(B) Tay-Sachs disease
(C) Hemophilia
(D) Xeroderma pigmentosum

2010

**239.** The horizontal gene transfer process requiring a specialized transducing phage is highly dependent on:
(A) The presence of an F plasmid
(B) Bacterial competence
(C) Formation of a sex pilus
(D) The exact site of prophage integration in the bacterial chromosome

2017

**240.** Arrange the typical life cycle sequence of a specialized transducing phage:
I. Precise integration at the attB site.
II. Induction and improper excision taking host genes.
III. Infection of an initial host cell.
IV. Lysis of the host and release of transducing phages.
V. Infection of a subsequent recipient cell and gene delivery.
(A) III → I → II → IV → V
(B) I → II → III → IV → V
(C) III → II → I → IV → V
(D) V → II → I → IV → III

2006

**241.** Fine-structure mapping demonstrated that crossing over can occur within a gene. Recombination between two mutant alleles in trans can reconstruct a:
(A) Wild-type allele
(B) Defective gene
(C) Completely deleted gene
(D) Pseudogene

2015

**242.** Phage-mediated genetic mapping relies on the frequency of co-transduction. Two genes are more likely to be co-transduced if they are:
(A) Located on different chromosomes
(B) Separated by more than 100 kilobases
(C) Closely linked on the bacterial chromosome

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(D) Completely unrelated functionally

2019

**243.** In fungi, the parasexual cycle does not involve standard meiotic segregation but still generates recombinant haploid progeny through:
(A) Mitotic crossing over followed by haploidization
(B) Extensive somatic mutation
(C) Viral infection and reassortment
(D) Transformation by naked DNA

2018

**244.** During tetrad analysis, an ascus showing a 2:4:2 segregation pattern is indicative of:
(A) First division segregation
(B) Second division segregation
(C) Complete lack of crossing over
(D) Gene duplication

2008

**245.** An unlinked gene pair analyzed in Neurospora crassa tetrads will statistically yield Parental Ditypes (PD) and Non-Parental Ditypes (NPD) in a ratio approximately equal to:
(A) 1:1
(B) 2:1
(C) 1:0
(D) 4:1

2017

**246.** Retroviral integrase fundamentally functions similarly to the transposase of which transposable elements?
(A) Class I Retrotransposons
(B) Group I Introns
(C) Class II DNA transposons
(D) Spliceosomes

2007

**247.** The Ac (Activator) element in maize provides which essential component allowing the Ds (Dissociation) element to transpose?
(A) Reverse transcriptase
(B) LTR sequences
(C) RNA polymerase
(D) A functional transposase enzyme

2017

**248.** Eukaryotic Class I transposable elements utilize an RNA intermediate, linking their evolutionary origin closely to:
(A) Retroviruses
(B) Bacteriophages
(C) Plasmids
(D) Prions

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2018

**249.** Transposition can result in:
(A) Precise DNA repair
(B) Chromosome breakage and mutable phenotypes
(C) Increased transcription of all cellular genes
(D) Telomere lengthening

2021

**250.** Simple Insertion Sequences (IS elements) are strictly confined to which genetic role?
(A) Carrying antibiotic resistance genes
(B) Encoding only the proteins required for their own transposition
(C) Mediating conjugation
(D) Forming viral capsids

**********

# ANSWERS

**1.** (A) The unit of genetic function
Seymour Benzer introduced the term cistron to define the functional unit of heredity. This genetic unit corresponds to a segment of DNA that encodes a single polypeptide or a functional RNA molecule. The cistron was identified through complementation tests, where two mutations in the same cistron fail to restore the wild-type phenotype in a trans configuration.

**2.** (C) Alternative splicing and overlapping genes
The traditional one gene-one polypeptide hypothesis suggested each gene produces a single protein. However, alternative splicing allows multiple mRNA variants to be produced from a single primary transcript. Additionally, overlapping genes utilize different reading frames within the same DNA sequence. These mechanisms enable a single genomic locus to encode several distinct polypeptides, fundamentally challenging the original concept.

**3.** (A) Mutation and recombination units, respectively.
Genetic units defined by Seymour Benzer include the muton and the recon. The muton represents the smallest structural unit of DNA capable of undergoing a mutation, which is essentially a single nucleotide pair. In contrast, the recon is the smallest unit within which genetic recombination can occur. Both terms highlight the discrete molecular nature of genetic material and processes.

**4.** (A) I and II
Non-coding RNAs are essential genetic elements that are transcribed from the DNA template but are not translated into functional proteins. This category includes structural molecules like transfer RNA and ribosomal RNA, which are fundamental to protein synthesis. Unlike protein-coding transcripts, these RNAs perform diverse catalytic and structural roles within the cellular machinery across all domains of biological life.

**5.** (B) Bacteriophage $\phi$ X174
The discovery of overlapping genes provided evidence that genomic space can be used with high efficiency. Bacteriophage $\phi$ X174 was the first organism whose genome demonstrated this phenomenon. By utilizing different reading frames or alternative initiation sites, this small virus encodes several proteins within a limited DNA sequence. This organization is particularly common in viral genomes with space constraints.

**6.** (B) 10.4
The B-DNA double helix is the most common physiological conformation of genetic material. According to the Watson-Crick model, this right-handed helix contains approximately 10.4 base pairs per complete turn. This value represents the average helical twist under standard biological conditions. The precise spacing ensures stability and allows for specific interactions between the DNA molecule and various regulatory proteins.

**7.** (D) A-DNA
DNA can adopt different conformations depending on its environment and hydration state. A-DNA is a right-handed helical form that is favored under conditions of low hydration or dehydration. It is characterized by having 11 base pairs per turn, making it shorter and wider than the standard B-form. This structure is often observed in double-stranded RNA and DNA-RNA hybrids.

**8.** (A) Left-handed helix with a zig-zag backbone
Z-DNA is a unique structural variant of the double helix characterized by a left-handed twist. Unlike the smooth backbone of the B-form, Z-DNA features a zig-zag phosphate backbone. This conformation typically arises in sequences with alternating purines and pyrimidines, such as repeating GC sequences. It is thought to play a role in regulating gene expression and relieving torsional strain.

**9.** (B) Prokaryotic DNA is located in a membrane-bound nucleus.
Prokaryotic and eukaryotic cells exhibit significant differences in their genomic organization. While eukaryotic DNA is enclosed within a double-membrane-bound nucleus, prokaryotic DNA is located in a specialized region called the nucleoid, which lacks a surrounding membrane. Furthermore, eukaryotic DNA is linear and

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2022

**174.** Hydroxylamine is a specific chemical mutagen that exclusively reacts with:
(A) Adenine
(B) Guanine
(C) Thymine
(D) Cytosine

2022

**175.** The Ames test utilizes auxotrophic strains of Salmonella typhimurium to assess the mutagenic potential of chemical compounds. The primary reversion measured is from:
(A) $Trp^-$ to $Trp^+$
(B) $His^-$ to $His^+$
(C) $Lac^-$ to $Lac^+$
(D) $Amp^R$ to $Amp^S$

2002

**176.** Ionizing radiation causes high lethality primarily by inducing:
(A) Thymine dimers
(B) Depurination
(C) Frameshift mutations
(D) Double-strand DNA breaks

2002

**177.** Site-directed mutagenesis using PCR frequently utilizes DpnI endonuclease to digest the parental DNA template. DpnI selectively targets:
(A) Methylated DNA (specifically $G^{\{m\}}ATC$ sequences)
(B) Unmethylated DNA
(C) Single-stranded RNA
(D) Okazaki fragments

2013

**178.** In cassette mutagenesis, if the inserted synthetic sequence contains a frameshift, the downstream consequence is typically:
(A) A silent mutation
(B) Enhanced expression
(C) A premature stop codon (nonsense mutation)
(D) Splicing interference

2002

**179.** Oligonucleotide-directed mutagenesis requires the synthesis of a mutagenic primer that is:
(A) Complementary except for the targeted mismatch(es)
(B) Completely complementary to the template
(C) Entirely random
(D) Composed exclusively of RNA

2003

**180.** Select the correct order of events in the Base Excision Repair (BER) pathway:
(A) AP endonuclease cuts backbone → DNA glycosylase removes base → Polymerase fills gap → Ligase seals
(B) DNA glycosylase removes base → AP endonuclease cuts backbone → Polymerase fills gap → Ligase seals
(C) Helicase unwinds → Polymerase fills gap → DNA glycosylase removes base → Ligase seals
(D) AP endonuclease cuts backbone → Helicase unwinds → Ligase seals → DNA glycosylase removes base

2012

**181.** In the Nucleotide Excision Repair (NER) system of E. coli, the initial recognition of bulky DNA damage (such as a pyrimidine dimer) is performed by:
(A) MutS
(B) UvrAB complex
(C) RecA
(D) DNA photolyase

2003

**182.** The SOS response is induced when extensive DNA damage results in the accumulation of:
(A) Double-stranded DNA loops
(B) Excess RNA polymerase
(C) Single-stranded DNA
(D) Unmethylated GATC sequences

2004

**183.** Which SOS response polymerase is highly error-prone and permits translesion synthesis across damaged templates?
(A) DNA Polymerase I
(B) DNA Polymerase II
(C) DNA Polymerase III
(D) DNA Polymerase V (UmuD'2C complex)

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2018

**184.** Non-homologous end joining (NHEJ) frequently results in:
(A) Small insertions or deletions at the joining site
(B) Perfect fidelity restoration
(C) Large chromosomal translocations universally
(D) Conversion of linear DNA to circular DNA

2010

**185.** Conjugation between an F' strain and an F- strain typically produces:
(A) A purely Hfr cell
(B) A partially diploid (merodiploid) cell
(C) Death of the recipient

**19.** (A) Chromosome end protection from degradation
Telomeres are specialized nucleoprotein complexes located at the ends of eukaryotic linear chromosomes. They consist of repetitive DNA sequences that protect the chromosome from degradation and prevent the cell's repair machinery from recognizing the ends as double-strand breaks. This protective cap is vital for maintaining genomic stability. Additionally, telomeres solve the end-replication problem by providing a buffer during DNA synthesis cycles.

**20.** (A) Both A and R are true and R is the correct explanation of A.
Eukaryotic DNA replication is semi-discontinuous, requiring RNA primers to initiate synthesis. Because DNA polymerase can only add nucleotides in a 5' to 3' direction, the extreme ends of the lagging strand cannot be fully replicated after the final primer is removed. This end-replication problem results in the progressive shortening of linear chromosomes in somatic cells, eventually leading to cellular senescence.

**21.** (B) DNA Polymerase I
During prokaryotic DNA replication, RNA primers used to initiate synthesis must be removed and replaced with DNA. DNA Polymerase I performs this critical task through its unique 5' to 3' exonuclease activity. After removing the ribonucleotides, the enzyme uses its polymerase activity to fill the resulting gap with the appropriate deoxyribonucleotides, ensuring the continuity and integrity of the newly synthesized strand.

**22.** (C) DNA Polymerase $\varepsilon$
Eukaryotic DNA replication utilizes several specialized polymerases to ensure high fidelity and efficiency. DNA Polymerase epsilon is the primary enzyme responsible for the continuous synthesis of the leading strand. It possesses high processivity and intrinsic proofreading capabilities. In contrast, DNA Polymerase delta is mainly involved in lagging strand synthesis, while DNA Polymerase $\alpha$ works with primase to initiate the synthesis process.

**23.** (D) Tus
Termination of replication in the circular Escherichia coli chromosome occurs at specific sequences called ter sites. These sites are located opposite the origin of replication. The Tus protein binds to these sequences and acts as a counter-helicase, blocking the progression of the replication fork in one direction. This mechanism ensures that the two forks meet and terminate within a designated region.

**24.** (A) Binding to the replication origin to initiate DNA synthesis
The initiation of DNA replication in eukaryotic cells begins with the binding of the Origin Recognition Complex to specific genomic sites. This multi-subunit complex serves as a scaffold for the assembly of the pre-replicative complex during the G1 phase. By marking the locations where replication will start, the complex ensures that the entire genome is duplicated exactly once during the S-phase.

**25.** (B) 5' to 3'
DNA synthesis is a fundamental biological process governed by the catalytic properties of DNA polymerases. These enzymes can only add new deoxyribonucleotides to the 3' hydroxyl group of an existing polynucleotide chain. Consequently, the growth of the new DNA strand always proceeds in the 5' to 3' direction. This unidirectional growth dictates the continuous and discontinuous nature of leading and lagging strands.

**26.** (B) cDNA library
A cDNA library is a collection of DNA sequences representing the mRNA molecules expressed in a specific cell or tissue at a particular time. Since it is derived from mature mRNA using reverse transcriptase, it lacks non-coding introns and regulatory sequences found in genomic DNA. These libraries are invaluable for studying gene expression patterns and for producing recombinant proteins in bacterial systems.

**27.** (A) Reverse transcriptase
The construction of a cDNA library begins with the isolation of total mRNA from a biological sample. The first essential enzymatic step involves the use of reverse transcriptase. This enzyme synthesizes a complementary DNA strand using the mRNA as a template. This process converts the transient and unstable RNA information into a stable DNA format that can be cloned into various vectors.

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**100.** (B) I, II, III and V
Tetrad analysis provides detailed information about genetic linkage and meiotic events. If Parental Ditypes equal Non-Parental Ditypes, the genes are unlinked. However, an excess of PD over NPD indicates genetic linkage. Second Division Segregation patterns are specifically used for centromere mapping. Tetratypes occur when there is a crossover between one gene and its centromere. This type of analysis is common in eukaryotes.

**101.** (B) Genetic reassortment
Genetic reassortment is a specialized form of recombination that occurs in viruses with segmented genomes. When two different viral strains infect the same host cell, the individual genomic segments can be reshuffled during the assembly of new viral particles. This process results in progeny with unique combinations of genetic material from both parent viruses. It is a major driver of viral evolution and new strains.

**102.** (C) Influenza A virus
The Influenza A virus is a prominent human pathogen that utilizes genetic reassortment to increase its diversity. Because its genome consists of eight separate RNA segments, co-infection of a single host with different strains can lead to the production of novel pandemic viruses. This process, often referred to as antigenic shift, can suddenly change the virus's surface proteins, making it unrecognizable.

**103.** (D) Copy-choice or template switching
Retroviruses, such as HIV, exhibit a unique form of recombination during the process of reverse transcription. This mechanism, known as copy-choice or template switching, occurs when the reverse transcriptase enzyme jumps from one RNA template to another during DNA synthesis. This frequent switching between the two co-packaged RNA genomes allows the virus to generate highly recombinant DNA progeny, contributing to its rapid evolution and resistance.

**104.** (A) E. coli B only
Seymour Benzer's landmark fine-structure mapping utilized rapid-lysis mutants of the T4 bacteriophage. He characterized these rII mutants based on their distinct plaque morphologies and host ranges. Specifically, rII mutants are unable to form plaques on E. coli strains carrying a lambda prophage, such as K12($\lambda$). However, they can still infect and form large, clear plaques on E. coli B, allowing precise mapping.

**105.** (B) In different cistrons
In Benzer's genetic analysis, the complementation test was used to determine if two rII mutations were located in the same or different functional units. When two different mutant phages co-infected E. coli K12($\lambda$), the formation of plaques indicated that the phages could provide the functions missing in each other. This result meant the mutations were in different cistrons, while a lack of plaques indicated the same.

**106.** (A) Insertion Sequence (IS)
The simplest type of transposable element found in prokaryotes is the Insertion Sequence. An IS element is a compact segment of DNA that contains only the information necessary for its own movement. It typically consists of a gene encoding the transposase enzyme flanked by short, inverted repeats. These repeats are recognized by the transposase during the excision and integration of the element into a new location.

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**107.** (A) Carry additional genes, such as antibiotic resistance genes, flanked by IS elements
Composite transposons are complex genetic elements that consist of a central region carrying additional genes, such as those for antibiotic resistance, flanked by two insertion sequences. The IS elements provide the transposase and the inverted repeats required for movement. This organization allows the entire segment, including the resistance genes, to transpose as a single unit, facilitating the rapid spread of drug resistance among bacterial populations.

**108.** (C) Cointegrate
Replicative transposition is a mechanism where the transposable element is duplicated, leaving the original copy intact while a new copy integrates elsewhere. This process involves the formation of a temporary fusion between the donor and target DNA molecules, called a cointegrate. The cointegrate contains two copies of the transposable element. It is eventually resolved into two separate molecules by a specific enzyme called resolvase.

**109.** (D) Tn10
Conservative transposition, often called the "cut-and-paste" mechanism, involves the physical

flexibility through mechanisms like alternative splicing.

**37.** (B) Group I introns
Introns are classified into different groups based on their splicing mechanisms. Group I introns are unique because they are capable of self-splicing without the aid of a protein-based spliceosome. This process requires a guanosine nucleotide cofactor, such as GMP or GTP. The 3' hydroxyl group of the free guanosine acts as a nucleophile to initiate the first transesterification reaction at the 5' splice site.

**38.** (C) snRNPs
The removal of introns from eukaryotic nuclear pre-mRNAs is carried out by a large ribonucleoprotein complex known as the spliceosome. The core functional components of this complex are small nuclear ribonucleoprotein particles, or snRNPs. Each snRNP consists of a small nuclear RNA molecule and several associated proteins. These particles recognize the splice sites and catalyze the biochemical reactions necessary for accurate intron excision.

**39.** (D) Lariat
During the splicing of spliceosomal introns, the reaction proceeds through a two-step transesterification process. In the first step, the 2' hydroxyl group of a specific adenine residue at the branch point attacks the 5' splice site. This creates a branched intermediate structure called a lariat, which is held together by an unusual 2'-5' phosphodiester bond. The lariat is subsequently released and degraded.

**40.** (B) I and III
Splicing mechanisms vary across different classes of introns. Group II introns utilize an internal adenine branch point to form a lariat structure during self-splicing, similar to the mechanism used by spliceosomes. Most spliceosomal introns in eukaryotic nuclei follow the GU-AG rule, where these dinucleotides mark the 5' and 3' boundaries, respectively. While less common, introns are also found in some prokaryotic genomes.

**41.** (B) Viral genomes
Overlapping genes are a genomic arrangement where two or more genes share a portion of the same DNA sequence. This organization is most frequently observed in viral genomes, where there is significant evolutionary pressure to minimize genome size while maximizing coding capacity. By utilizing different reading frames or initiation sites, viruses can encode a greater number of proteins within a very compact genetic space.

**42.** (A) Pseudogenes
Pseudogenes are genomic sequences that are related to functional genes but have lost their ability to code for proteins. They often contain mutations such as premature stop codons, frameshifts, or deletions that render them non-functional. Although they may be transcribed, they do not produce a functional product. These sequences provide important insights into the evolutionary history and duplication events of an organism's genome.

**43.** (A) Introns and functional promoters
A processed pseudogene is created when a mature mRNA molecule is reverse transcribed and integrated back into the genome. Because it originates from mRNA, it typically lacks the introns and the original functional promoter found in the parental gene. Instead, it often contains a poly-A track at its 3' end. These features distinguish processed pseudogenes from those formed through genomic DNA duplication events.

**44.** (C) Coding multiple sequences in compact spaces
The primary evolutionary advantage of overlapping genes is the maximization of coding efficiency. This organization allows an organism to store more genetic information within a smaller physical amount of DNA. This is particularly crucial for viruses and small bacteria, where packaging constraints limit the size of the genome. By using overlapping sequences, these organisms can produce a diverse set of proteins from limited genetic material.

**45.** (D) Use of different reading frames
The phenomenon of overlapping genes was famously demonstrated in the bacteriophage $\phi$ X174. Researchers found that this virus utilizes different reading frames within the same DNA sequence to encode multiple proteins. For instance, the sequence for one gene might contain the starting codon for another gene in a different frame. This efficient use of genetic information is essential for the virus's compact lifecycle.

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**64.** (D) Depurination
DNA molecules are susceptible to spontaneous chemical damage even under normal physiological conditions. Depurination occurs when the covalent bond between a purine base, such as adenine or guanine, and the deoxyribose sugar is broken by hydrolysis. This process leaves an apurinic site in the DNA backbone. If these sites are not repaired before replication, they can lead to base substitutions or deletions.

**65.** (A) Transversion
Base substitution mutations are categorized into two types: transitions and transversions. A transition occurs when a purine is replaced by another purine, or a pyrimidine by another pyrimidine. A transversion, however, involves the replacement of a purine with a pyrimidine or vice versa. Therefore, changing an adenine (a purine) to a cytosine (a pyrimidine) is a specific example of a transversion mutation.

**66.** (C) Intercalating agents
Chemical mutagens interact with DNA in various ways to induce genetic changes. Intercalating agents, such as proflavin or ethidium bromide, are flat, hydrophobic molecules that can slide between adjacent base pairs in the double helix. This intercalation distorts the DNA structure, causing DNA polymerase to add or delete nucleotides during replication. As a result, these agents are primarily known for inducing frameshift mutations.

**67.** (A) Pyrimidine dimers (e.g., thymine dimers)
Ultraviolet radiation is a potent physical mutagen that causes specific types of DNA damage. When DNA is exposed to UV light, adjacent pyrimidine bases, such as two thymines, can form covalent bonds with each other. These pyrimidine dimers distort the double helix and block the progression of DNA polymerase during replication. If left unrepaired, these lesions can lead to mutations and cancer.

**68.** (D) Intercalating agents
Several chemicals are used in laboratory settings due to their ability to bind to DNA. Ethidium bromide and acridine orange are classic examples of intercalating agents. These molecules possess a planar ring structure that allows them to wedge themselves between the stacked base pairs of the DNA double helix. This binding property is utilized for staining DNA in gels, but it also makes them mutagenic.

**69.** (B) Guanine
5-bromouracil is a base analog that chemically resembles thymine. It can be incorporated into DNA during replication in place of the normal base. While it usually pairs with adenine, 5-bromouracil frequently shifts into its rare enol tautomer. In this form, it pairs with guanine instead of adenine. This mispairing leads to a transition mutation, specifically changing an AT base pair to a GC.

**70.** (A) Both A and R are true and R is the correct explanation of A.
Ethyl methanesulfonate is a powerful alkylating agent used extensively in genetic research to induce mutations. It works by adding an ethyl group to the O-6 position of guanine. The resulting O-6-ethylguanine base-pairs with thymine instead of cytosine during DNA replication. This chemical modification leads to a high frequency of GC to AT transition mutations, making it an effective tool for forward genetic screens.

**71.** (D) Intentional changes in known DNA sequences
Site-directed mutagenesis is a fundamental technique in molecular biology that allows researchers to introduce specific, intentional changes into a known DNA sequence. Unlike random mutagenesis, this method targets a precise location within a gene to alter a single nucleotide or a small group of bases. This approach is invaluable for studying the function of proteins, analyzing regulatory sequences, and engineering enzymes with improved properties.

**72.** (A) Specially designed mutagenic primers
In the overlap extension method of site-directed mutagenesis, two separate PCR reactions are performed using specially designed mutagenic primers. These primers contain the desired sequence change and overlap with each other. After the initial amplification, the resulting DNA fragments are mixed and extended in a second PCR step. This process creates a full-length product that carries the mutation, which can then be cloned.

**73.** (B) Unique flanking restriction endonuclease sites
Cassette mutagenesis is a technique used to replace a specific segment of a gene with a

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**55.** (D) High tryptophan levels enable rapid translation of the leader peptide
In the tryptophan operon, the leader mRNA can form different hairpin structures. When intracellular tryptophan levels are high, the ribosome moves quickly through the leader peptide sequence. This allows regions 3 and 4 of the mRNA to base-pair and form a terminator hairpin. This structure signals RNA polymerase to stop transcription before it reaches the structural genes, thereby conserving cellular energy and resources.

**56.** (A) RNA Polymerase II
Eukaryotic cells contain three primary RNA polymerases, each responsible for transcribing different types of genes. RNA Polymerase II is the enzyme specifically tasked with the synthesis of all protein-coding genes. It transcribes DNA into messenger RNA precursors, as well as several small nuclear and microRNAs. The activity of this polymerase is tightly regulated by a wide array of transcription factors and promoter elements.

**57.** (B) 5'-5' triphosphate linkage
The processing of eukaryotic mRNA begins with the addition of a 7-methylguanosine cap to the 5' end of the transcript. This modification is unique because it involves an unusual 5'-5' triphosphate linkage between the guanosine and the first nucleotide of the RNA. This cap is essential for protecting the mRNA from exonucleases and is recognized by the cellular machinery for translation initiation.

**58.** (B) AAUAAA
The termination of eukaryotic transcription is closely linked to the polyadenylation of the 3' end of the mRNA. This process is triggered by a specific consensus sequence in the pre-mRNA, which is most commonly AAUAAA. Recognition of this signal by specialized proteins leads to the cleavage of the transcript and the subsequent addition of a long tail of adenine residues by poly(A) polymerase.

**59.** (A) TFIIH
Initiation of eukaryotic transcription involves the assembly of a pre-initiation complex. The general transcription factor TFIIH plays a pivotal role in the transition from initiation to elongation. It possesses kinase activity that phosphorylates the C-terminal domain of RNA Polymerase II. This chemical modification causes a conformational change that allows the polymerase to break free from the promoter and begin synthesizing the RNA transcript.

**60.** (A) P-2, Q-1, R-4, S-3
Eukaryotic cells utilize distinct RNA polymerases for different genetic tasks. RNA Polymerase I is dedicated to the synthesis of large ribosomal RNAs. RNA Polymerase II transcribes protein-coding genes into mRNA. RNA Polymerase III is responsible for producing transfer RNAs and the 5S ribosomal RNA. Additionally, plants possess specialized polymerases involved in the synthesis of small interfering RNAs, highlighting the complexity of eukaryotic transcription.

**61.** (D) Tautomeric shifts
Spontaneous mutations often arise from the inherent chemical properties of nitrogenous bases. Tautomeric shifts occur when a base transiently converts into a rare structural isomer. For example, thymine may switch from its common keto form to a rare enol form. These shifts change the hydrogen-bonding patterns of the bases, leading to improper base pairing during DNA replication and causing permanent genetic changes.

**62.** (C) Thymine
Chemical modifications to DNA can lead to spontaneous mutations. Cytosine is frequently methylated at the 5-position to form 5-methylcytosine, which plays a role in gene regulation. However, 5-methylcytosine can undergo spontaneous deamination, a process that removes an amino group and converts the base into thymine. Because thymine is a natural component of DNA, this error is often missed by cellular repair systems.

**63.** (C) Insertions or deletions (frameshifts)
Replication slippage is a common spontaneous mutation mechanism that occurs primarily in regions of the genome containing short tandem repeats. During DNA synthesis, the template and the newly synthesized strand may temporarily misalign. This misalignment causes DNA polymerase to either skip bases or add extra ones, resulting in small insertions or deletions. Such mutations are often responsible for frameshifts and the expansion of repeats.

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**28.** (C) Gene isolation via mutant phenotype restoration
Functional complementation is a powerful screening technique used to identify and isolate specific genes from a DNA library. It involves introducing the library into a mutant host organism that lacks a particular function. If a cloned DNA fragment restores the wild-type phenotype, it indicates that the fragment contains the functional version of the gene, allowing researchers to pinpoint the sequence of interest.

**29.** (D) BAC (Bacterial Artificial Chromosome)
For the construction of genomic libraries containing large eukaryotic DNA fragments, Bacterial Artificial Chromosomes are the preferred vectors. They are based on the F-plasmid of Escherichia coli and can stably accommodate inserts exceeding 100 kilobases. Their large capacity makes them ideal for physical mapping and large-scale sequencing projects, as they provide better coverage of the genome compared to smaller plasmid or phage vectors.

**30.** (B) I and III
Genomic libraries contain the entire genetic content of an organism, including exons, introns, and non-coding regulatory elements. In contrast, cDNA libraries are tissue-specific and reflect the transcriptome of a cell at a given moment. These libraries are generated by reverse transcribing mRNA into DNA. Therefore, while genomic libraries are uniform across tissues, cDNA libraries vary significantly depending on the cell type and environment.

**31.** (B) Specific DNA sequences
Southern blotting is a classic molecular biology technique used to detect the presence of specific DNA sequences within a complex mixture. It involves the separation of DNA fragments by gel electrophoresis, their transfer to a solid membrane, and subsequent hybridization with a labeled probe. This method is essential for applications such as gene mapping, detecting mutations, and identifying homologous sequences across different species.

**32.** (D) RNA instead of DNA
Northern blotting is a hybridization-based technique specifically designed for the detection and quantification of RNA molecules. While Southern blotting is utilized for DNA analysis, Northern blotting allows researchers to study gene expression patterns by measuring the size and abundance of specific transcripts. Both methods share similar steps, including electrophoresis and membrane transfer, but differ in the nature of the nucleic acid being investigated.

**33.** (C) 94-98° C
The polymerase chain reaction is a cyclical process used to amplify specific DNA sequences. The first step in each cycle is denaturation, which involves heating the sample to a high temperature, typically between 94 and 98 degrees Celsius. This heat breaks the hydrogen bonds between complementary base pairs, resulting in the separation of the double-stranded DNA into two individual template strands for synthesis.

**34.** (C) Binding to targets for complementary detection
In hybridization-based detection methods, a probe is a short, labeled sequence of single-stranded DNA or RNA. Its primary function is to bind specifically to a complementary target sequence within a sample. By forming a stable double-stranded molecule through base pairing, the probe allows for the precise identification and localization of the sequence of interest, which is then detected using radioactive or fluorescent signals.

**35.** (A) Taq DNA polymerase
The success of the polymerase chain reaction relies on the use of a thermally stable DNA polymerase. Taq polymerase, isolated from the bacterium Thermus aquaticus, is the most widely used enzyme for this purpose. It can withstand the high temperatures required for DNA denaturation without losing its catalytic activity. This stability allows the reaction to proceed through multiple cycles without the need for replenishment.

**36.** (D) Exons and introns
Many eukaryotic genes are organized as split genes, meaning their coding sequences are interrupted by non-coding segments. The coding regions, which are retained in the mature messenger RNA and eventually translated into proteins, are called exons. The non-coding intervening sequences that are removed during the RNA processing stage are known as introns. This organization allows for increased genetic

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synthetic DNA fragment. This method requires the target DNA to have two unique restriction endonuclease sites flanking the region to be mutated. The original segment is removed by digestion and replaced with a double-stranded oligonucleotide "cassette" that contains the desired sequence changes, allowing for precise and efficient modifications.

**74.** (A) Uracil residues in place of thymine
The Kunkel method is a classic technique for oligonucleotide-directed mutagenesis that utilizes a specialized template DNA. The template is grown in a bacterial strain that incorporates uracil instead of thymine. When the mutant strand is synthesized in vitro using a mutagenic primer, it contains only normal bases. Upon transformation into a wild-type host, the uracil-containing template strand is selectively degraded, strongly favoring the replication of the mutant strand.

**75.** (A) Risks of unintended polymerase error mutations
While PCR-based site-directed mutagenesis is highly effective, it has certain limitations. One primary concern is the potential for introducing unintended mutations elsewhere in the gene or plasmid. This occurs because even high-fidelity DNA polymerases can make errors during the multiple cycles of amplification. Therefore, it is standard practice to sequence the entire cloned fragment after mutagenesis to ensure that only the desired change is present.

**76.** (C) Photolyase
Photoreactivation is a direct DNA repair mechanism that targets pyrimidine dimers caused by ultraviolet light. This process is mediated by the enzyme photolyase, which binds specifically to the damaged site. When activated by visible light, the enzyme uses the energy to break the covalent bonds between the adjacent pyrimidines, restoring them to their original state. This mechanism is common in many organisms, although not in humans.

**77.** (D) DNA glycosylases
Base excision repair is a critical pathway for correcting non-bulky DNA damage, such as deaminated or oxidized bases. The process is initiated by DNA glycosylases, a family of enzymes that recognize and remove specific damaged bases. Each glycosylase is specific to a particular type of lesion. By cleaving the glycosidic bond, the enzyme creates an apurinic or apyrimidinic site, which is then processed by other repair proteins.

**78.** (C) Methylated at specific sequence motifs
The mismatch repair system in Escherichia coli must distinguish between the correct template strand and the newly synthesized strand containing the error. This discrimination is based on the methylation state of specific GATC sequences. After replication, there is a short delay before the new strand is methylated by Dam methylase. The MMR system recognizes the unmethylated state of the new strand and targets it for repair.

**79.** (B) LexA
The SOS response is a global regulatory network in bacteria that is activated by severe DNA damage. Under normal conditions, the expression of SOS genes is repressed by the LexA protein, which binds to specific promoter sequences. When damage occurs, the RecA protein becomes activated and promotes the self-cleavage of LexA. This relief of repression allows for the high-level expression of DNA repair and translesion synthesis enzymes.

**80.** (B) I only
Various pathways maintain genomic integrity. Nucleotide excision repair is specialized for removing bulky lesions that distort the DNA helix. Homologous recombination provides an accurate, error-free mechanism for repairing double-strand breaks by using a sister chromatid as a template. In contrast, non-homologous end joining is an inherently error-prone process that often results in small insertions or deletions at the site where the DNA ends are rejoined.

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**81.** (A) Sex pilus
Bacterial conjugation is a method of horizontal gene transfer that involves the direct movement of DNA from a donor cell to a recipient cell. This process requires physical contact, which is mediated by a specialized surface appendage called the sex pilus. The donor cell produces the pilus to recognize and attach to a suitable recipient, eventually drawing the two cells together to facilitate DNA transfer.

**82.** (C) It becomes F+.
In a standard mating between an F+ donor cell and an F- recipient cell, the entire F plasmid is typically transferred. The process begins with a single-stranded nick in the plasmid, followed by rolling circle replication. As the single strand enters the recipient, it is circularized and converted back into double-stranded DNA. Consequently, the recipient cell acquires the F plasmid and becomes a fertile F+ cell.

**83.** (A) The integration of the F plasmid into the bacterial chromosome
High frequency of recombination strains are a specialized type of donor cell in Escherichia coli. They are characterized by the integration of the F plasmid into the main bacterial chromosome through a recombination event. Because the plasmid is part of the chromosome, Hfr cells can transfer chromosomal genes to a recipient during conjugation. The transfer usually begins at the integrated plasmid's origin of transfer.

**84.** (D) F'
An F' plasmid is created through an imprecise excision event from an Hfr chromosome. During this process, the integrated F factor is excised along with a segment of the adjacent bacterial DNA. This results in a specialized plasmid that carries specific chromosomal genes. When an F' donor cell conjugates with a recipient, it transfers these bacterial genes, creating a partially diploid cell known as a merodiploid.

**85.** (B) Mapping the bacterial chromosome in minutes
Interrupted mating experiments were pioneered to map the bacterial chromosome. By using Hfr strains and stopping the conjugation process at different time intervals, researchers could determine the order in which chromosomal genes were transferred to the recipient. This technique allowed for the construction of a genetic map measured in minutes, providing early evidence that the Escherichia coli chromosome is a large, circular molecule.

**86.** (C) Uptake of naked exogenous DNA from the environment
Bacterial transformation is a process of horizontal gene transfer in which a cell takes up naked, exogenous DNA from its surrounding environment. This DNA can originate from dead or lysed cells. Once inside the recipient cell, the acquired genetic material can be integrated into the host genome through recombination or maintained as a plasmid, potentially providing the organism with new phenotypic traits.

**87.** (D) Competent
Not all bacterial cells are capable of taking up DNA from their environment. A cell that possesses the physiological state necessary for transformation is described as competent. This state involves the expression of specialized proteins that facilitate DNA binding and transport across the cell membrane. Competence can be a natural part of an organism's lifecycle or can be induced artificially in the laboratory.

**88.** (A) Calcium chloride treatment and heat shock
In many laboratory procedures, bacteria like Escherichia coli must be made competent through artificial means. One common method involves treating the cells with a cold calcium chloride solution followed by a brief heat shock. The calcium ions neutralize the negative charges on the DNA and the cell membrane, while the heat shock creates temporary pores, allowing the DNA to enter the cell for transformation.

**89.** (D) Single-stranded DNA
During the process of natural transformation in bacteria such as Bacillus subtilis, the mechanism of DNA uptake is highly regulated. As double-stranded DNA binds to the cell surface, specialized membrane proteins degrade one of the strands. Consequently, the genetic material typically enters the cytoplasm as single-stranded DNA. This single-stranded molecule can then participate in homologous recombination with the host's chromosome to be permanently incorporated.

**90.** (A) I and II
Homologous integration is a key step in the permanent acquisition of new genetic material during transformation. This process requires significant sequence similarity between the incoming DNA and the host chromosome. In bacteria like Escherichia coli, this recombination event is primarily mediated by the RecA protein, which promotes strand exchange. Successful integration results in a stable genetic change, rather than the formation of a plasmid.

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**91.** (A) Transduction
Transduction is a form of horizontal gene transfer in which bacterial DNA is moved from one cell to another by a bacteriophage. During the viral infection cycle, errors in packaging can lead to the inclusion of host genomic DNA within the newly formed viral particles. When these phages infect a new host, they inject the donor bacterial DNA, which can then integrate into the recipient's genome.

**92.** (C) Random packaging of host DNA into phage heads
Generalized transduction occurs when a bacteriophage mistakenly packages a random fragment of host bacterial DNA into its capsid during the lytic cycle. This error occurs because the viral packaging machinery occasionally fails to distinguish between viral and host DNA based on size. As a result, any gene from the donor bacterial chromosome has an equal chance of being transferred to a new recipient cell.

**93.** (D) Transfers only specific host genes flanking the prophage integration site
Specialized transduction is a highly specific process mediated by temperate bacteriophages. It occurs during the transition from a lysogenic to a lytic cycle when a prophage is excised imprecisely from the host chromosome. Because the excision is flawed, specific host genes flanking the integration site are packaged along with the viral DNA. Consequently, only a limited set of chromosomal genes can be transferred.

**94.** (B) The relative map distance between two closely linked genes
Co-transduction frequency is a valuable parameter used in bacterial genetics to determine the relative distance between two genes on a chromosome. Since a bacteriophage can only package a limited amount of DNA, two genes must be located close together to be included in the same viral particle. The higher the frequency with which two genes are co-transduced, the closer they are in physical distance.

**95.** (C) Lambda phage
The lambda phage of Escherichia coli is the classic example of a virus that performs specialized transduction. It integrates its DNA into the bacterial chromosome at a specific site called attB, which is located between the gal and bio genes. Errors during the excision of the prophage lead to the creation of transducing particles that specifically carry either the galactose or biotin operons.

**96.** (D) Meiosis and standard sexual structures
The parasexual cycle is a non-sexual mechanism for genetic recombination found in certain fungi. It allows these organisms to achieve genetic diversity through processes such as plasmogamy, karyogamy, and mitotic crossing over, followed by haploidization. Notably, this cycle occurs entirely without the involvement of meiosis or the formation of specialized sexual structures. This is particularly important for fungi that lack a known sexual phase.

**97.** (A) Aspergillus nidulans
The parasexual cycle was first discovered and characterized by G. Pontecorvo and his colleagues in the fungus Aspergillus nidulans. This organism served as an excellent model for studying genetic recombination outside of the typical sexual lifecycle. The discovery provided a framework for understanding how many asexual fungi maintain genetic variation and adapt to changing environments, challenging the idea that meiosis is the only source.

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**98.** (C) Twin spots
Mitotic recombination in heterozygous fungi can occasionally lead to the formation of homozygous recessive cells. In organisms with visible phenotypic markers, this event can result in adjacent patches of tissue showing different recessive traits, a phenomenon known as twin spots. These spots are a direct visual result of reciprocal crossing over between homologous chromosomes during mitosis, followed by the specific segregation of chromatids.

**99.** (A) Linear, allowing the tracing of first and second division segregation
Tetrad analysis is a powerful genetic tool, particularly in fungi like Neurospora crassa. This organism produces linear asci, where the eight ascospores are arranged in a precise order that reflects their meiotic history. This arrangement allows researchers to distinguish between first and second division segregation patterns. By analyzing the position of different alleles within the ascus, scientists can accurately map the distance between genes.

**46.** (B) -25 to -30 bp
The TATA box is a highly conserved DNA sequence found within the core promoter of many eukaryotic genes. It serves as a primary binding site for the TATA-binding protein, which initiates the assembly of the transcription pre-initiation complex. In most eukaryotes, this element is located approximately 25 to 30 base pairs upstream of the transcription start site, where it helps position RNA polymerase II.

**47.** (B) Enhancer
Enhancers are regulatory DNA sequences that significantly increase the transcription rate of associated genes. They possess unique characteristics that distinguish them from core promoters. Specifically, enhancers can function effectively regardless of their distance from the start site, and they remain functional even when their orientation is reversed. They work by binding specific activator proteins that interact with the basal transcription machinery via DNA looping.

**48.** (D) DNA loops mediated by protein complexes
Enhancers often act at great distances from the gene they regulate. To facilitate interaction with the basal transcription machinery at the promoter, the intervening DNA must be bent. This process involves the formation of large DNA loops, which are stabilized by protein complexes such as mediators and cohesins. These loops bring distant activator proteins into direct physical contact with the general transcription factors and polymerase.

**49.** (A) Specific physiological signals like hormones or heat shock
Response elements are specific DNA sequences located within the promoters of certain genes. They allow for the coordinate regulation of multiple genes in response to specific physiological or environmental signals, such as hormones, metals, or heat shock. When a signal is received, specialized transcription factors bind to these elements, ensuring that all genes required for a specific cellular response are activated simultaneously.

**50.** (B) I, II and V
Eukaryotic gene regulation involves several key elements. Core promoters are essential for assembling the pre-initiation complex, while silencers bind repressor proteins to downregulate transcription. The CAAT box is another regulatory element that influences the frequency of transcription initiation. These elements work together with enhancers, which can be located far from the gene, and response elements to ensure precise control over gene expression levels.

**51.** (A) lacI
The lac operon in Escherichia coli is regulated by a repressor protein that prevents transcription when lactose is absent. This repressor is encoded by the lacI gene, which is located upstream of the operon and is constitutively expressed. The repressor binds to the operator site, physically blocking RNA polymerase from moving forward and transcribing the structural genes involved in lactose metabolism.

**52.** (D) CRP (CAP) - cAMP complex
The lac operon is subject to positive regulation, which ensures the cell preferentially uses glucose. When glucose levels are low, the concentration of cyclic AMP increases. cAMP binds to the catabolite activator protein (CAP or CRP), forming a complex that binds to a specific site near the lac promoter. This binding facilitates the attachment of RNA polymerase, significantly increasing the rate of transcription initiation.

**53.** (B) The coupling of transcription and translation in prokaryotes
Regulation of the tryptophan operon in bacteria includes a mechanism called attenuation, which fine-tunes expression based on the availability of charged tRNA molecules. Attenuation depends on the fact that transcription and translation are coupled in prokaryotes, occurring simultaneously in the cytoplasm. The movement of the ribosome along the leader peptide sequence directly determines whether the nascent mRNA will form a terminator or anti-terminator structure.

**54.** (C) Both a positive and negative regulator depending on arabinose availability
The arabinose operon is regulated by the AraC protein, which acts as both an activator and a repressor. In the absence of arabinose, AraC binds to specific sites and forms a DNA loop that prevents transcription. When arabinose is present, it binds to AraC, causing a conformational change that allows the protein to act as a positive regulator, stimulating the recruitment of RNA polymerase.

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excision of the transposable element from its original site and its subsequent integration into a new target location. Unlike replicative transposition, no cointegrate intermediate is formed, and the total number of element copies in the genome remains constant. The bacterial transposon Tn10 is a well-studied example that utilizes this direct movement strategy.

**110.** (A) P-3, Q-4, R-1, S-2
Transposable elements rely on several key features for mobility. Inverted repeats are specific sequences at the ends of the element recognized by excision enzymes. Transposase is the primary enzyme responsible for cutting and rejoining the DNA. Resolvase is required in replicative transposition to break down the cointegrate intermediate. Finally, direct repeats are host DNA sequences that are duplicated upon insertion.

**111.** (C) Retrotransposons
Eukaryotic transposable elements are divided into two main classes. Class I elements, known as retrotransposons, replicate using a "copy-and-paste" mechanism that involves an RNA intermediate. The element is first transcribed into RNA, which is then converted back into DNA by the enzyme reverse transcriptase. This newly synthesized DNA copy is integrated into a new genomic site, allowing the element to increase its total copy number.

**112.** (D) Zea mays (Maize)
Barbara McClintock discovered the first transposable elements while studying the genetics of kernel pigmentation in Zea mays, or maize. She identified two interacting factors: the autonomous Activator (Ac) element and the non-autonomous Dissociation (Ds) element. This groundbreaking work revealed that genes are not fixed in place but can move within the genome, leading to unstable mutations and diverse phenotypic patterns in the kernels.

**113.** (A) Transposase mutation requiring Ac assistance
In the maize transposable element system, the Ds element is classified as non-autonomous because it cannot move on its own. It typically contains mutations in the gene encoding the transposase enzyme, rendering it non-functional. For Ds to transpose, a functional Ac element must be present in the same cell to provide the necessary transposase. This enzyme recognizes the inverted repeats of the Ds element.

**114.** (C) Non-LTR retrotransposons
Long Interspersed Nuclear Elements are a major component of the human genome, making up about 21% of its content. These elements are classified as non-LTR retrotransposons because they lack the long terminal repeats found in other retrotransposons. LINEs encode their own reverse transcriptase and endonuclease, allowing them to move via an RNA intermediate. The LINE-1 family is the only group capable of active transposition.

**115.** (A) A female lacking P elements mates with a male carrying P elements.
Hybrid dysgenesis in Drosophila melanogaster is a syndrome of genetic abnormalities that occurs when P elements become active in the germline. This phenomenon is observed when a female lacking P elements is mated with a male carrying them. Because the female's egg lacks the cytoplasmic repressors needed to silence transposition, the P elements from the sperm mobilize uncontrollably, causing mutations and sterility.

**116.** (D) Alternative splicing
The discovery of alternative splicing has fundamentally revised our understanding of the gene. This process allows a single pre-mRNA transcript to be processed in different ways by selectively including or excluding exons. Consequently, a single gene can produce multiple distinct functional proteins, known as isoforms. This mechanism significantly expands the protein diversity of eukaryotic organisms without requiring a corresponding increase in total gene number.

**117.** (B) siRNA
Small interfering RNAs are a class of non-coding RNA molecules that play a central role in the process of RNA interference. These double-stranded RNA fragments are produced by the enzyme Dicer and then incorporated into the RNA-induced silencing complex. By guiding the complex to perfectly complementary messenger RNA molecules, siRNAs trigger the cleavage and degradation of the target, silencing the expression of specific genes.

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**155.** (A) Opposite strands of the DNA in the same locus
Overlapping genes represent a highly efficient genomic organization where the same DNA sequence encodes multiple products. These genes can be arranged in several ways, including being transcribed from opposite strands of the double helix within the same locus. In such cases, the sense strand for one gene serves as the template for the other, allowing for complex regulatory interactions and reduction.

**156.** (C) Multi-protein encoding within 3.2 kb
The Hepatitis B virus possesses an extremely compact circular genome of only about 3.2 kilobases. Despite this small size, the virus encodes several essential proteins by utilizing an extensive system of overlapping genes. Nearly every nucleotide in the HBV genome is used to code for more than one protein by employing different reading frames. This organization is a remarkable evolutionary adaptation.

**157.** (D) Retrotransposition of mature mRNA
Pseudogenes are classified into two main types based on their mode of origin. Non-processed pseudogenes arise from duplication events and often retain introns. In contrast, processed pseudogenes are formed through a mechanism involving the retrotransposition of mature mRNA. A spliced mRNA molecule is reverse transcribed into DNA and integrated back into the genome. Consequently, these pseudogenes are characterized by a lack.

**158.** (D) Nucleolus
In eukaryotic cells, the production of ribosomal RNA is a highly specialized process that occurs within a distinct sub-compartment of the nucleus called the nucleolus. This region contains the genes for several rRNAs, which are organized in large tandem arrays. These genes are transcribed by RNA Polymerase I to produce a single large precursor molecule, which is then processed into subunits.

**159.** (B) Sp1
The GC box is a common regulatory element found in the promoters of many eukaryotic housekeeping genes. It consists of the consensus sequence GGGCGG and is often located upstream of the transcription start site. This element is specifically recognized and bound by the transcription factor Sp1. The binding of Sp1 to the GC box helps to recruit the general transcription machinery.

**160.** (A) P-4, Q-3, R-2, S-1
Eukaryotic gene expression is controlled by several regulatory elements. Enhancers bind activator proteins to upregulate transcription, while silencers bind regressors to downregulate it. The TATA box is a core promoter element that mediates the assembly of the basal transcription complex. Finally, insulators are specialized sequences that block the interaction between an enhancer and a non-target promoter, ensuring regulatory signal specificity.

**161.** (A) Can function effectively in either orientation
Enhancers and promoters are both essential for gene regulation but differ in their fundamental properties. Unlike core promoters, which must be located at a precise distance and orientation relative to the transcription start site, enhancers are highly flexible. They can function effectively from thousands of base pairs away and remain functional regardless of their orientation, communicating with the promoter via DNA looping.

**162.** (C) TBP (TATA-binding protein)
The initiation of eukaryotic transcription by RNA polymerase II requires the assembly of several general transcription factors. The process begins with the binding of TFIID to the promoter. TFIID is a large multi-subunit complex that includes the TATA-binding protein. The TBP subunit is specifically responsible for recognizing and binding to the TATA box sequence, which providing a scaffold for the machinery.

**163.** (A) Zinc-finger containing steroid hormone receptors
Glucocorticoid Response Elements are specific DNA sequences found in the promoters of genes regulated by steroid hormones. These elements are recognized by the glucocorticoid receptor, a specialized transcription factor. When the hormone binds to its receptor, the complex binds to the GRE. The receptor contains zinc-finger motifs that facilitate sequence-specific binding to the DNA, thereby stimulating the transcription of the target genes.

**164.** (D) Glucose is low and lactose is present
The lac operon in Escherichia coli is designed to ensure that the enzymes for lactose metabolism

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**127.** (B) Proteins that destroy closely related bacterial strains
Col plasmids are a class of extrachromosomal genetic elements that provide a competitive advantage to their bacterial hosts. They encode bacteriocins, such as colicins in Escherichia coli, which are toxic proteins that kill or inhibit the growth of closely related bacterial strains. In addition to the toxin gene, these plasmids also carry an immunity gene to protect the host cell from its own bacteriocin.

**128.** (D) The oriC locus
DNA replication in the Escherichia coli chromosome is a highly coordinated process that begins at a single, specific location known as the oriC locus. This origin of replication contains conserved sequence motifs that are recognized by the DnaA initiator protein. The binding of DnaA leads to the localized unwinding of the DNA double helix, creating a replication bubble and recruiting the replication machinery.

**129.** (A) The par system and interactions with the cell membrane/septum
After replication, the two circular bacterial chromosomes must be accurately segregated into the daughter cells. This process is facilitated by the par system, which consists of specific DNA sequences and proteins that act like a primitive mitotic apparatus. These proteins ensure that the replicated DNA is actively moved to opposite poles of the cell, often through interactions with the developing cell septum.

**130.** (A) I and II
The nucleosome is the primary unit of DNA packaging in eukaryotes. It consists of approximately 147 base pairs of DNA wrapped nearly twice around a core octamer of histone proteins. This core is composed of two molecules each of histones H2A, H2B, H3, and H4. Histone H1 is not part of the core but binds to the outside, helping to stabilize the structure.

**131.** (A) Is permanently condensed and predominantly localized at centromeres and telomeres
Heterochromatin is highly condensed, transcriptionally inactive DNA. It is categorized into two types: constitutive and facultative. Constitutive heterochromatin remains permanently condensed in all cell types of an organism. It is predominantly found at structural regions such as centromeres and telomeres. These regions consist mainly of repetitive DNA sequences and play essential roles in chromosome stability and segregation, rather than encoding proteins.

**132.** (B) Serve as an attachment point for the kinetochore complex during segregation
The centromere is a specialized region of the eukaryotic chromosome essential for accurate genetic segregation during cell division. It contains specific DNA sequences that serve as the assembly site for the kinetochore, a complex protein structure. During mitosis and meiosis, spindle fibers attach to the kinetochore, allowing the cell to pull sister chromatids or homologous chromosomes to opposite poles, ensuring correct segregation.

**133.** (D) Reverse transcriptase carrying its own RNA template
Telomerase is a unique enzyme responsible for maintaining the ends of linear chromosomes. Biochemically, it is a specialized reverse transcriptase that carries its own RNA template. The enzyme uses this internal RNA sequence to synthesize complementary DNA repeats at the 3' ends of telomeres. This process compensates for the progressive loss of DNA during replication cycles, preventing chromosome shortening and maintaining lifespan.

**134.** (C) Homo sapiens
In many multicellular organisms, including humans, telomerase activity is tightly regulated. While the enzyme is active in germ cells and stem cells, it is absent or downregulated in most adult somatic cells. As a result, the telomeres in these cells shorten each time the cell divides. This progressive loss of genetic material acts as a biological clock, eventually triggering cellular senescence.

**135.** (D) Okazaki fragments
DNA replication is an asymmetric process because polymerases can only synthesize DNA in the 5' to 3' direction. While the leading strand is synthesized continuously, the lagging strand is produced in a series of small, discontinuous segments. Each segment, called an Okazaki fragment, requires its own RNA primer. Once synthesized, these fragments are eventually joined together by DNA ligase to form a complete strand.

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the term "recon" to describe the smallest element of the genome within which recombination can occur. Benzer demonstrated that recombination could take place between mutations located extremely close together, showing that the recon is physically equivalent to a nucleotide pair.

**194.** (B) Shift
Recombination in viruses with segmented genomes can lead to sudden genetic changes. When two different viral strains co-infect the same cell, their genomic segments can be reshuffled during assembly, a process called reassortment. This mechanism is responsible for antigenic shift, which involves the emergence of a new virus with a different combination of surface proteins, often leading to potential pandemic strains.

**195.** (A) Reverse Transcriptase
Retroviruses like HIV maintain high genetic diversity through a unique recombination mechanism. Because each viral particle contains two copies of its RNA genome, the reverse transcriptase enzyme can switch between these two templates during DNA synthesis. This "copy-choice" process is facilitated by the enzyme's relatively low processivity and its ability to re-initiate synthesis on a different RNA molecule during transcription.

**196.** (D) Species lacking a known sexual cycle (Deuteromycetes)
The parasexual cycle is a significant source of genetic variation in certain groups of fungi. While sexual reproduction involves meiosis, the parasexual cycle achieves recombination through mitosis. This process is particularly vital for fungi classified as Deuteromycetes, which have no known sexual stage in their life cycle. By allowing for chromosome exchange through cell fusion, the cycle enables these organisms to adapt.

**197.** (B) Much lower than meiotic crossing over
Crossing over is a process that reshuffles genetic material between homologous chromosomes. While it is a routine and frequent event during prophase I of meiosis, it can also occur during mitosis in some organisms, such as fungi in the parasexual cycle. However, the frequency of mitotic crossing over is significantly lower than that of meiotic crossing over in most species.

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**198.** (B) Progressive, random chromosome loss (haploidization)
In the fungal parasexual cycle, genetic recombination is followed by a process that restores the original haploid state. This is not achieved through meiosis but through a phenomenon called haploidization. In a diploid cell, errors during mitosis can lead to aneuploidy. These unstable cells undergo a progressive and random loss of individual chromosomes until they reach a stable and recombinant haploid state.

**199.** (D) Absence of crossing over at the centromere
Tetrad analysis in linear fungi like Neurospora allows researchers to track the segregation of alleles during meiotic divisions. A first division segregation pattern is observed when the two different alleles are separated into opposite halves of the ascus in a 4:4 arrangement. This pattern occurs when there is no crossing over between the gene locus and the centromere of the chromosome.

**200.** (A) First division segregation
In the octads produced by Neurospora crassa, the arrangement of the eight ascospores provides direct evidence of meiotic events. The most common arrangement is the 4:4 pattern, where four spores of one parental genotype are followed by four of the other. This diagnostic pattern is a hallmark of first division segregation, indicating the two alleles were separated during the first division.

**201.** (B) Holliday model and mismatch repair of heteroduplex DNA
During tetrad analysis, researchers occasionally observe unusual segregation ratios, such as 6:2 or 5:3, instead of the expected 4:4. This phenomenon is known as gene conversion. It provides strong molecular evidence for the Holliday model of recombination. Gene conversion occurs when a heteroduplex DNA region, containing mismatched base pairs from two different parental strands, is repaired by the mismatch repair machinery.

**202.** (B) Jumping genes
Transposable elements are specialized segments of DNA that have the unique ability to move from one location to another within a genome. Because of this mobility, they are frequently referred to by the colloquial term "jumping

the necessary resolution for distinguishing DNA pieces of varying lengths. After separation, the DNA pattern is transferred to a solid membrane for further analysis, allowing for sequence-specific detection in the mixture.

**146.** (D) The denaturation of double-stranded DNA to allow probe hybridization
Before transferring DNA from an electrophoresis gel to a membrane in Southern blotting, the gel is typically treated with an alkaline solution. This step serves to denature the double-stranded DNA fragments into single strands. Denaturation is essential because it allows the DNA to bind effectively to the membrane and ensures that the target sequences are available for hybridization with a single-stranded probe.

**147.** (A) Expressed mRNA sequences translated into cDNA
Reverse Transcription Polymerase Chain Reaction is a specialized technique designed to amplify and study expressed gene sequences. It begins by using the enzyme reverse transcriptase to convert a sample's messenger RNA into complementary DNA. This cDNA then serves as the template for a standard PCR amplification. RT-PCR is widely used to measure gene expression levels and detect specific transcripts in a sample.

**148.** (D) Measuring the amplification of DNA continuously during the exponential phase
Quantitative Real-Time PCR represents a major advancement over standard PCR techniques. While traditional PCR only allows for end-point analysis, qPCR continuously monitors the amplification of DNA during the exponential phase of the reaction. This is achieved through the use of fluorescent dyes. By measuring the signal in real time, researchers can accurately quantify the initial amount of target DNA in the sample.

**149.** (B) Northern blotting
DNA microarrays are powerful tools that allow for the simultaneous analysis of thousands of genes. This technology fundamentally expands upon the principles of Northern blotting by immobilizing a vast number of specific DNA probes on a solid surface. When a fluorescently labeled cDNA sample is hybridized to the array, the signal at each spot reflects the expression level of the corresponding target gene.

**150.** (B) I, II and V
Split genes are a hallmark of eukaryotic genomes, consisting of coding exons and non-coding introns. Splicing is carried out by the spliceosome, a complex of snRNAs and proteins. In this process, the branch point sequence is essential for the formation of a lariat intermediate. While some introns like Group I are self-splicing, the GU-AG rule typically defines the boundaries of nuclear introns.

**151.** (D) GU and AG
The accuracy of the splicing process depends on the recognition of specific consensus sequences at the intron-exon boundaries. In most eukaryotic nuclear pre-mRNAs, the 5' splice site is defined by an invariant GU dinucleotide, while the 3' splice site ends with an invariant AG. These sequences are part of a larger conserved motif recognized by the spliceosome, ensuring precise excision of the intron.

**152.** (A) Sxl (Sex-lethal)
Alternative splicing plays a central role in the sex determination pathway of Drosophila melanogaster. The Sex-lethal gene is a key regulator that undergoes alternative splicing to produce different protein products in males and females. In females, the Sxl protein facilitates the production of more functional Sxl protein through a feedback loop, whereas in males, an exon containing a premature stop codon.

**153.** (B) The 3'-OH of a free Guanosine nucleotide
Group I introns are characterized by a unique self-splicing mechanism that does not involve a lariat structure. Instead, the process is initiated by an exogenous guanosine nucleotide, such as GTP, which acts as a cofactor. The 3' hydroxyl group of this free guanosine molecule serves as a nucleophile, attacking the phosphodiester bond at the 5' splice site, transferring the guanosine to the intron.

**154.** (A) Caenorhabditis elegans
While most splicing occurs within a single RNA transcript, some organisms utilize trans-splicing, where exons from two separate RNA molecules are joined together. This process is particularly common in the nematode Caenorhabditis elegans. In these organisms, a specialized leader sequence is added to the 5' ends of many different messenger RNAs. This mechanism is essential for the maturation of certain gene transcripts.

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**174.** (D) Cytosine
Hydroxylamine is a highly specific chemical mutagen that reacts with only one of the four DNA bases. It specifically modifies cytosine by adding a hydroxyl group to its amino group. This modification changes the chemical properties of the base so that it pairs with adenine instead of guanine, leading to a transition mutation where a CG base pair is converted.

**175.** (B) $His^-$ to $His^+$
The Ames test is a widely used biological assay for assessing the mutagenic potential of chemical compounds. It utilizes special strains of the bacterium Salmonella typhimurium that carry mutations in the genes required for histidine biosynthesis. The test measures the ability of a substance to induce mutations that restore the bacteria's ability to grow on histidine-free media, known as His+ reversion.

**176.** (D) Double-strand DNA breaks
Ionizing radiation, such as X-rays, is a potent physical mutagen that can cause severe damage to biological molecules. Unlike UV light, which primarily induces pyrimidine dimers, ionizing radiation has enough energy to knock electrons out of atoms, creating reactive free radicals. These radicals cause various lesions, but the most lethal form of damage is the induction of double-strand breaks in DNA.

**177.** (A) Methylated DNA (specifically $G^{\{m\}}ATC$ sequences)
In protocols for site-directed mutagenesis, the enzyme DpnI is used to increase the efficiency of the process. This restriction endonuclease is unique because it only cleaves DNA that is methylated at the N-6 position of adenine within $G^mATC$ sequences. Since the parental template DNA is typically isolated from an E. coli strain that performs this methylation, DpnI selectively digests the template.

**178.** (C) A premature stop codon (nonsense mutation)
Cassette mutagenesis involves replacing a segment of a gene with a synthetic oligonucleotide. If the inserted "cassette" sequence contains an error that shifts the reading frame, it will alter every subsequent codon. In many cases, this frameshift will eventually encounter a triplet that functions as a stop signal in the new frame. This leads to the production of a truncated protein.

**179.** (A) Complementary except for the targeted mismatch(es)
Oligonucleotide-directed mutagenesis relies on the synthesis of a short primer used to initiate DNA replication on a template strand. To introduce a specific change, the primer is designed to be complementary to the target sequence except for one or more nucleotides at the desired site of mutation. During the synthesis reaction, the primer binds to the template despite the small mismatch.

**180.** (B) DNA glycosylase removes base → AP endonuclease cuts backbone → Polymerase fills gap → Ligase seals
Base excision repair is a multi-step pathway used to fix non-bulky DNA damage. The process begins with a DNA glycosylase that recognizes and removes the damaged base, leaving an abasic site. Next, an AP endonuclease cleaves the sugar-phosphate backbone. DNA polymerase then fills the resulting gap with the correct nucleotide. Finally, DNA ligase seals the nick in the backbone, completing repair.

**181.** (B) UvrAB complex
Nucleotide excision repair is the primary system for correcting bulky DNA lesions. In Escherichia coli, the process is initiated by the UvrABC proteins. The UvrA and UvrB subunits form a complex that scans the DNA for structural distortions. Once a lesion is identified, UvrA dissociates, and UvrB recruits the UvrC endonuclease to make precise cuts on both sides of damage.

**182.** (C) Single-stranded DNA
The SOS response is an emergency repair system in bacteria that is activated when the genome suffers extensive damage. The primary signal that triggers this response is the accumulation of single-stranded DNA. This occurs when DNA polymerase stalls at a lesion while the helicase continues to unwind the double helix. The excess single-stranded DNA is quickly coated by the RecA protein.

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**183.** (D) DNA Polymerase V (UmuD'2C complex)
When a bacterial cell encounters severe DNA damage that blocks normal replication, it may utilize specialized SOS polymerases to bypass the lesions. DNA Polymerase V is a highly error-prone enzyme that performs translesion synthesis. Unlike high-fidelity polymerases, it

are produced only when necessary. Significant transcription occurs only under two specific conditions: lactose must be present to inactivate the lac repressor, and glucose must be absent to allow high levels of cAMP. The presence of cAMP enables the CAP-cAMP complex to bind near the promoter.

**165.** (B) Allolactose
The lac operon is an inducible system that is normally kept in a repressed state. When lactose is present in the environment, a small amount is converted into an isomer called allolactose by the enzyme β-galactosidase. Allolactose acts as the true inducer of the operon by binding to the lac repressor protein. This binding causes a conformational change that reduces repression.

**166.** (C) Tryptophan
The tryptophan operon in bacteria is a repressible system that regulates the synthesis of enzymes for tryptophan production. The trp repressor protein is normally inactive and cannot bind to the operator site. However, when intracellular levels of tryptophan are high, the amino acid itself acts as a co-repressor. Tryptophan binds to the repressor, changing its shape and allowing it to bind.

**167.** (B) Tryptophan
Attenuation of the trp operon is a regulatory mechanism that senses the availability of tryptophan through translation. The leader sequence of the mRNA contains a small open reading frame encoding a leader peptide. This peptide includes two tandem tryptophan codons. If tryptophan levels are low, the ribosome stalls at these codons, which allows the mRNA to form an anti-terminator structure.

**168.** (A) araI1 and araO2
The arabinose operon is regulated by the AraC protein through a mechanism involving DNA looping. In the absence of arabinose, AraC molecules bind to two widely separated sites: the araI1 site and the araO2 site. By binding to both locations simultaneously, the AraC proteins interact, causing the intervening DNA to bend into a loop. This structural change prevents RNA polymerase access.

**169.** (D) Poly(A) polymerase (PAP)
The final stage of eukaryotic transcription involves the processing of the 3' end of the mRNA. After RNA polymerase II transcribes the polyadenylation signal, the nascent transcript is cleaved by a specific endonuclease. Following this cleavage, the enzyme poly(A) polymerase adds a string of adenine nucleotides to the new 3' end. This tail is essential for mRNA stability and nuclear export.

**170.** (A) Both A and R are true and R is the correct explanation of A.
The 5' cap is a critical modification of eukaryotic messenger RNA that serves multiple functions. It is recognized by the cap-binding complex and translation initiation factors like eIF4E, which are necessary for the recruitment of the ribosome to the mRNA. Furthermore, the cap provides essential protection against degradation by 5' to 3' exonucleases, ensuring the stability of the genetic transcript.

**171.** (A) Adenine
Depurination is a spontaneous chemical event that results in the loss of a purine base from the DNA backbone, leaving an abasic site. If this damage is not repaired before the DNA is replicated, it poses a challenge for DNA polymerase. In many cases, the polymerase preferentially inserts an adenine residue opposite the blank site. This leads to a base substitution.

**172.** (C) Cytosine
Tautomeric shifts are spontaneous changes in the chemical structure of nitrogenous bases that can lead to mutations. Adenine normally exists in its amino form and pairs with thymine. However, it can transiently shift into its rare imino tautomer. In this rare state, the hydrogen-bonding pattern of adenine is altered such that it pairs incorrectly with cytosine instead of thymine during replication.

**173.** (D) Replication slippage
Certain genetic disorders, such as Huntington's disease, are caused by the expansion of trinucleotide repeats within specific genes. The primary mechanism responsible for this expansion is replication slippage. During DNA synthesis, the newly formed strand can detach and reattach incorrectly in a repetitive region, causing a loop to form. When this loop is repaired, it results in an increased number of repeat units.

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**136.** (B) β-clamp (sliding clamp)
For DNA polymerases to synthesize long stretches of DNA efficiently, they must remain attached to the template. This is achieved by sliding clamp proteins that encircle the DNA. In prokaryotes, this function is performed by the β-clamp subunit of DNA Polymerase III. In eukaryotes, the functionally identical protein is called Proliferating Cell Nuclear Antigen. PCNA acts as a processivity factor during replication.

**137.** (C) DNA ligase
During DNA replication, the RNA primers are removed and replaced with deoxyribonucleotides, leaving a small break or "nick" in the sugar-phosphate backbone. DNA ligase is the enzyme responsible for sealing these nicks. It catalyzes the formation of a phosphodiester bond between the 3' hydroxyl group of one fragment and the 5' phosphate group of the next, ensuring the continuity of the DNA.

**138.** (A) The extreme length of linear chromosomes
Eukaryotic genomes are significantly larger and more complex than those of prokaryotes. To ensure that the entire genome is replicated within a reasonable time frame, eukaryotic chromosomes contain multiple origins of replication. This allows for the simultaneous initiation of DNA synthesis at many different sites along the linear DNA molecule. Without these origins, replicating a single human chromosome would take far too long.

**139.** (A) Helicase
In the replication machinery of Escherichia coli, the DnaB protein plays a fundamental role as the primary helicase. It is a hexameric enzyme that uses energy from ATP hydrolysis to unwind the double-stranded DNA at the replication fork. By breaking the hydrogen bonds between complementary bases, DnaB creates the single-stranded templates necessary for synthesis. It also interacts with primase to coordinate.

**140.** (B) I only
Genomic and cDNA libraries serve different research purposes. A genomic library represents the entire DNA sequence of an organism and is identical regardless of the tissue it was derived from. In contrast, cDNA libraries are tissue-specific as they are generated from mRNA. Therefore, the statement that genomic libraries vary between tissues is incorrect, as the DNA sequence remains constant throughout the individual.

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**141.** (D) Stuffer substitution with 10-20 kb inserts
Phage lambda replacement vectors are highly efficient tools for constructing genomic libraries. These vectors are engineered with a removable central segment, often called a stuffer fragment. This segment can be replaced with large pieces of foreign DNA, typically between 10 and 20 kilobases in length. The resulting recombinant phages are then packaged and used to infect bacteria, allowing for stable storage and screening.

**142.** (C) Radiolabeled or fluorescently labeled DNA/RNA probe
Colony hybridization is a technique used to identify specific DNA sequences within a bacterial library. Bacterial colonies are grown on a plate and then transferred to a solid membrane. The cells are lysed and the DNA is denatured and fixed to the membrane. The presence of the target sequence is detected by incubating the membrane with a labeled DNA or RNA probe.

**143.** (C) Heterologous probing
Screening a DNA library often involves finding a sequence that is similar to one already known. Using a probe derived from the DNA of one species to identify the corresponding gene in a different species is called heterologous probing. This approach relies on the evolutionary conservation of gene sequences. For example, a mouse gene can be used to isolate the human equivalent.

**144.** (D) Isolating overlapping fragments to map regions
Chromosome walking is a mapping technique used to isolate a specific gene when its approximate location is known but a nearby marker is already cloned. The process involves using the end of a known DNA fragment as a probe to identify an overlapping fragment from a genomic library. By repeating this step, researchers can "walk" along the chromosome until they reach the target gene.

**145.** (A) Agarose gel
Southern blotting begins with the digestion of genomic DNA into smaller fragments using restriction endonucleases. These fragments are then separated by size through electrophoresis on an agarose gel. The agarose matrix provides

can incorporate nucleotides opposite damaged bases, such as pyrimidine dimers. While this process prevents a complete stall, it frequently introduces various mutations.

**184.** (A) Small insertions or deletions at the joining site
Non-homologous end joining is a pathway used by eukaryotic cells to repair double-strand breaks by directly rejoining the broken ends of the DNA. Because this process does not utilize a homologous template for repair, it is inherently error-prone. The ends are often processed by nucleases before they are ligated together, which frequently results in the loss or gain of nucleotides.

**185.** (B) A partially diploid (merodiploid) cell
An F' plasmid is a fertility factor that has been excised from the bacterial chromosome along with a segment of host DNA. When an F' donor cell mates with an F- recipient cell, it transfers the entire plasmid, including the integrated bacterial genes. Because the recipient now possesses two copies of these specific genes, the resulting cell is described as partially diploid.

**186.** (A) Encoding the pilin protein and assembly machinery for the sex pilus
The F-plasmid of Escherichia coli is a large extrachromosomal element that enables the cell to act as a genetic donor. Its ability to transfer itself to other cells is dependent on the tra genes, which constitute a large operon. These genes encode all the components necessary for conjugation, including the pilin protein for the assembly of the sex pilus.

**187.** (A) A single circular map
Early genetic research using Hfr interrupted mating provided crucial evidence about the structure of the bacterial genome. By observing the order and time at which different genes were transferred to a recipient cell, scientists were able to construct a genetic map. These studies consistently showed that the markers could be arranged into a single, continuous circular loop, establishing a circular chromosome.

**188.** (B) Competence stimulating peptide (CSP)
Natural competence for transformation is a highly regulated process in many bacteria, such as Streptococcus pneumoniae. The development of the competent state is triggered by a quorum-sensing mechanism. The bacteria synthesize and secrete a signaling molecule called the Competence Stimulating Peptide. As the population density increases, the concentration of CSP rises until it reaches a threshold level, triggering transformation genes.

**189.** (D) gal
Specialized transduction is a form of genetic transfer mediated by temperate phages like lambda. These phages integrate into the bacterial chromosome at a specific site. In E. coli, lambda integrates between the gal and bio loci. When the prophage is induced to enter the lytic cycle, it may undergo an improper excision event that captures a neighboring bacterial gene, such as gal.

**190.** (A) V → I → IV → II → III
The process of specialized transduction begins when a temperate phage infects a host and its DNA integrates into the chromosome. Later, the prophage is induced to enter the lytic cycle. During this transition, an improper excision event occurs, where some host DNA is taken. These hybrid molecules are packaged and released. Finally, the resulting transducing phage infects a recipient host.

**191.** (B) Any gene in the bacterial chromosome
Transduction is the phage-mediated transfer of bacterial DNA, categorized into generalized and specialized types. The primary difference lies in which parts of the host genome can be moved. In generalized transduction, any random segment of the bacterial chromosome can be packaged into a phage head. In contrast, specialized transduction is restricted to transferring only those specific host genes flanking the integration site.

**192.** (D) Unusually large, clear plaques
Plaque morphology is an important phenotypic trait used to study bacteriophage genetics. Rapid-lysis or r mutants of the T4 phage were famously used by Seymour Benzer. These mutants are defective in the process that normally delays host cell lysis during a viral infection. As a result, r mutants cause the bacteria to burst much sooner, forming plaques that are unusually large.

**193.** (A) A single nucleotide pair
In his groundbreaking work on the fine structure of the gene, Seymour Benzer redefined the classical concept of genetic units. He introduced

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**118.** (D) Neurospora crassa
The "one gene-one enzyme" hypothesis was formulated by George Beadle and Edward Tatum based on their research with the bread mold Neurospora crassa. By inducing mutations and screening for auxotrophs that could not synthesize specific nutrients, they demonstrated that each mutation affected a single metabolic step. This work established a direct link between genes and biochemical reactions, laying the foundation for modern genetics.

**119.** (D) Intron sequences
Retrotransposed or processed pseudogenes are created from mature mRNA, which has already undergone splicing. Consequently, these sequences are easily distinguished from their functional counterparts because they completely lack introns. Additionally, they often possess a poly-A tail and are usually located far from the original gene locus. Because they lack a functional promoter, they are typically not expressed, though they may accumulate mutations.

**120.** (A) Both A and R are true and R is the correct explanation of A.
Alternative splicing is a critical regulatory mechanism in eukaryotes that allows a single gene to encode multiple protein products. By including different combinations of exons in the mature mRNA, the cell can produce various isoforms with distinct functions. This process greatly enhances the complexity of the proteome and allows for tissue-specific or developmental regulation, explaining why complex organisms exist with relatively few genes.

**121.** (B) An increase in UV absorbance at 260 nm
When double-stranded DNA is denatured into single strands by heat or chemicals, it undergoes a phenomenon known as the hyperchromic shift. This is characterized by a significant increase in the absorption of ultraviolet light at a wavelength of 260 nm. The shift occurs because the unstacking of the nitrogenous bases in the single-stranded state allows them to absorb more light than the double helix.

**122.** (A) It offers a sterically accessible, sequence-specific interaction site for regulatory proteins.
The B-DNA double helix possesses two grooves of different sizes: the major groove and the minor groove. The major groove is wider and more accessible, exposing a unique pattern of chemical groups for each base pair sequence. This makes it the primary site for sequence-specific interactions with regulatory proteins. These proteins recognize and bind to the specific chemical signatures within the major groove to control activity.

**123.** (A) C-G
The melting temperature of DNA is the point at which half of the double-stranded molecules are denatured. This temperature is directly influenced by the base composition of the DNA. Specifically, cytosine-guanine base pairs are held together by three hydrogen bonds, compared to the two bonds in adenine-thymine pairs. Therefore, DNA sequences with a higher GC content are more stable and require more heat energy.

**124.** (C) A-DNA
While B-DNA is the standard form in cells, DNA can adopt other shapes under specific conditions. A-DNA is a right-handed helix that is more compact and wider than the B-form. It is generally considered a laboratory artifact because it forms primarily under conditions of high salt concentration or low hydration. However, its structure is biologically relevant as it closely resembles double-stranded RNA molecules.

**125.** (D) Negative supercoiling
Supercoiling is essential for the efficient packaging of the large bacterial chromosome into the small cell. Most DNA found in living organisms exists in a state of negative supercoiling. This under-winding of the double helix stores energy and facilitates the separation of the two strands, which is necessary for processes like transcription and replication. Topoisomerases, such as DNA gyrase, are responsible for this state.

**126.** (C) tra operon
For a plasmid to be self-transmissible through conjugation, it must contain a specific set of genes known as the tra operon. This region encodes all the proteins required for the process, including those needed for the assembly of the sex pilus and the DNA transfer machinery. The tra genes also regulate the timing and efficiency of transfer, ensuring that the plasmid can spread effectively.

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genes." First discovered by Barbara McClintock, these elements can influence gene expression, cause mutations, and drive genomic evolution in almost all biological domains of life.

**203.** (A) Recognition and binding sites for the transposase enzyme
The movement of simple transposable elements, such as Insertion Sequences, depends on specific DNA motifs at their boundaries. These elements are flanked by inverted repeats, which are sequences that read the same on opposite strands. The inverted repeats serve as the essential recognition and binding sites for the transposase enzyme, which catalyzes the excision of the element from its original genomic site.

**204.** (B) Makes staggered cuts in the target DNA, which are subsequently filled in by host DNA polymerase
Transposition events are almost always accompanied by the creation of direct repeats at the target site. This target site duplication occurs because the transposase enzyme makes staggered, asymmetrical cuts in the host DNA during integration. After the transposable element is inserted, the single-stranded regions are filled in by the host's DNA polymerase, resulting in the duplication of the sequence.

**205.** (D) Kernel pigmentation
The activity of transposable elements in maize was originally identified through their dramatic effects on the phenotype of kernel pigmentation. The movement of the Ds element into and out of genes responsible for pigment production leads to unstable mutations. This results in kernels with variegated patterns, such as spots or streaks, providing visual evidence that genetic elements could move on chromosomes.

**206.** (B) RNA
Retrotransposons are a major class of transposable elements that utilize a unique "copy-and-paste" mechanism for mobility. Unlike DNA transposons that move directly as DNA, retrotransposons are first transcribed into an RNA intermediate by the cell's RNA polymerase. This RNA is then converted back into a DNA copy by an enzyme called reverse transcriptase before integrating into a different genomic location.

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**207.** (B) SINEs (Short Interspersed Nuclear Elements)
The human genome contains a vast number of transposable elements, with the Alu family being the most abundant. These elements are approximately 300 base pairs long and are classified as Short Interspersed Nuclear Elements. Alu elements are non-autonomous retrotransposons, meaning they do not encode their own reverse transcriptase and rely on other elements, such as LINEs, for their mobility.

**208.** (D) Specific repressor proteins synthesized from alternatively spliced transposase transcripts
In wild-type populations of Drosophila melanogaster, the activity of P elements is strictly controlled to prevent genetic damage. In somatic tissues, the transposition of these elements is repressed by specific proteins produced through alternative splicing. The primary transcript can be processed to yield a repressor protein that binds to the element and prevents its mobilization, thereby protecting the organism's somatic genome.

**209.** (A) Transposase
Class II transposable elements move through the genome using a "cut-and-paste" mechanism. This process is governed by a single multi-functional enzyme called transposase. The transposase first recognizes the inverted repeats at the ends of the element and catalyzes its excision from the original DNA site. It then identifies a suitable target sequence and facilitates the integration of the DNA molecule.

**210.** (A) P-4, Q-2, R-1, S-3
Mutations can have various effects on the resulting protein sequence. A silent mutation does not alter the amino acid. A missense mutation results in the replacement of one amino acid with another. A nonsense mutation introduces a premature stop codon, leading to a truncated protein. Finally, a frameshift mutation alters the entire downstream reading frame, typically resulting in a non-functional polypeptide.

**211.** (A) One gene-multiple transcripts/isoforms
The classical definition of a gene as a single functional unit or cistron has been replaced by more complex models in modern genetics. The discovery of alternative splicing demonstrated that a single genomic locus can produce multiple distinct messenger RNA transcripts and protein

isoforms. This means that a gene is better defined as a region of DNA yielding functional products.

**212.** (D) Nucleotide sequence
Overlapping genes can be identified through the computational analysis of genomic sequences. Researchers look for regions where more than one open reading frame is present within the same stretch of DNA. If these different frames are found to encode functional protein domains, it indicates that the nucleotide sequence is used twice. This method is common in viral and bacterial genomic analysis.

**213.** (B) Inducing targeted mRNA degradation or translational repression
MicroRNAs are a class of small, non-coding RNA molecules that play a vital role in regulating gene expression in eukaryotes. They function by binding to partially complement sequences in the 3' untranslated regions of target messenger RNAs. This binding typically leads to either the degradation of the mRNA or the repression of its translation by the cellular ribosome machinery.

**214.** (B) Writhe (Wr)
The topological state of a DNA molecule is described by the equation $Lk = Tw + Wr$. In this formula, the term "writhe" refers to the number of times the double helix axis crosses over itself in three-dimensional space. This represents the supercoiling of the DNA molecule. Positive writhe occurs when DNA is over-wound, while negative writhe represents under-winding.

**215.** (D) Highly repetitive (satellite) DNA
Cot curve analysis is a technique used to study the complexity of genomic DNA by measuring the rate at which denatured strands renature. In this process, the fastest renaturing fraction represents sequences present in many copies throughout the genome. These are highly repetitive or satellite DNA sequences. Because there are so many copies, the chance of complementary strands meeting is high.

**216.** (A) Nucleoid-associated proteins (NAPs) and RNA/protein tethering
The bacterial genome is organized into a compact structure called the nucleoid. Within the nucleoid, circular DNA is divided into multiple independent supercoiled domains. This arrangement is maintained by nucleoid-associated proteins, such as HU, which help bend and anchor the DNA. Additionally, molecules of RNA and various proteins provide structural tethering to the cell membrane, ensuring that relaxation remains localized.

**217.** (B) Antisense RNA (RNA I)
The ColE1 plasmid is a widely used cloning vector that maintains a stable copy number through a sophisticated antisense RNA mechanism. Replication is initiated by the formation of an RNA primer called RNA II. However, the plasmid also produces a small antisense molecule known as RNA I. When plasmid levels are high, RNA I binds to RNA II, preventing replication initiation.

**218.** (B) Large arrays of tandemly repeated satellite DNA
Centromeres in higher eukaryotes are characterized by a unique genomic organization. These regions typically consist of vast arrays of tandemly repeated DNA sequences, often referred to as satellite DNA. For example, in humans, α-satellite DNA is the primary centromeric component. Although these sequences do not code for proteins, they are essential for centromere structural integrity and kinetochore assembly during division.

**219.** (A) TTAGGG
Telomeres are protective caps at the ends of linear chromosomes consisting of repetitive DNA sequences. In all vertebrates, the primary telomeric repeat is the hexanucleotide sequence TTAGGG. This sequence is repeated hundreds to thousands of times. The G-rich strand typically extends as a single-stranded overhang, which protects the chromosome ends from being recognized as DNA damage or undergoing enzymatic degradation.

**220.** (C) A is true but R is false.
Eukaryotic genomes are much larger than prokaryotic ones and must be replicated within the time of the S-phase. To achieve this, eukaryotes utilize thousands of origins of replication that fire simultaneously along each chromosome. However, eukaryotic DNA polymerases actually synthesize DNA significantly slower than their prokaryotic counterparts. Therefore, multiple origins are necessary to compensate for both genome size and speed.

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**221.** (D) Lagging strand looping to align polymerases
In Escherichia coli, the replication of the leading and lagging strands is coordinated by a protein complex called the replisome. Because the two strands are antiparallel and polymerase only works 5' to 3', the lagging strand template must be looped. This looping allows the two DNA polymerase III core enzymes to move in the same physical direction along the fork.

**222.** (B) Expression library screening
An expression library is a collection of cloned DNA fragments inserted into specialized vectors containing functional promoters and regulatory elements. For example, vectors may use a strong viral promoter, such as the T7 promoter, to drive transcription and translation in a host cell. This allows researchers to produce and screen for functional protein products based on their activity or biological properties.

**223.** (C) Functional complementation
Functional complementation is a strategic approach used to isolate a specific gene responsible for a known mutant phenotype. The process involves constructing a genomic or cDNA library from a wild-type organism and then introducing it into a mutant strain. If a specific DNA fragment can restore the wild-type phenotype in the host, it indicates that the fragment contains the functional gene.

**224.** (D) Restriction enzyme digestion and Southern blotting
Restriction Fragment Length Polymorphism analysis is a molecular technique used to detect variations in DNA sequences. It combines the use of restriction enzymes with Southern blotting. First, DNA is digested with a restriction endonuclease, which cuts at specific sites. These fragments are then separated by electrophoresis and identified using a labeled probe, allowing researchers to visualize polymorphisms used as genetic markers.

**225.** (B) Spliceosomal introns
Group II introns and spliceosomal introns are two classes of intervening sequences that share a remarkable evolutionary and mechanistic connection. Both types are excised through a two-step transesterification process that involves an internal adenine residue at a branch point. This reaction leads to the formation of a characteristic lariat intermediate. Modern eukaryotic spliceosomes are thought to have evolved from ancient introns.

**226.** (C) Splicing enhancers and silencers (exonic or intronic)
The process of alternative splicing is tightly regulated to ensure the correct protein isoforms are produced. This regulation is mediated by specific RNA-binding proteins that recognize short sequences within the pre-mRNA. These sequences, known as splicing enhancers and silencers, can either recruit or block the assembly of the spliceosome. By influencing exon inclusion, these proteins dictate the final protein structure.

**227.** (A) Expressed or "alive" pseudogene
While most pseudogenes are considered non-functional relics, some have regained biological significance. These are classified as expressed or "alive" pseudogenes. Although they may have lost the ability to encode a protein, they are still transcribed into long non-coding RNAs. These transcripts can perform regulatory roles, such as acting as molecular decoys to sequester microRNAs that would otherwise target genes.

**228.** (D) ATP hydrolysis
In eukaryotic cells, the tight packaging of DNA into nucleosomes can block the access of transcription machinery. To overcome this, cells utilize chromatin remodeling complexes that can restructure nucleosomes. These large protein complexes are molecular motors that utilize energy derived from ATP hydrolysis to break the bonds between DNA and histones. This activity exposes promoters, allowing RNA polymerase to initiate.

**229.** (B) Enhancers
In the yeast Saccharomyces cerevisiae, gene regulation often involves specific DNA sequences called Upstream Activating Sequences. These elements serve as binding sites for transcriptional activator proteins, which recruit the general transcription machinery to the promoter. UAS elements are functionally equivalent to enhancers. Like enhancers, they can act at a distance from the core promoter and are essential for regulated gene expression.

NODIA

**230.** (B) 5' Capping → Splicing → 3' Polyadenylation
In eukaryotes, the maturation of a primary RNA transcript into a functional messenger RNA involves several coordinated steps. The

III. Rho factor moves along the DNA template in a 3' to 5' direction.

Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2011

**31.** Which general transcription factor in eukaryotes is responsible for phosphorylating the C-terminal domain (CTD) of RNA Polymerase II?
(A) TFIIA
(B) TFIIB
(C) TFIID
(D) TFIIH

2018

**32.** What is the primary function of the TATA-binding protein (TBP) during eukaryotic pre-initiation complex assembly?
(A) It unwinds the DNA double helix for the polymerase
(B) It phosphorylates the polymerase C-terminal domain
(C) It binds the TATA box and induces a DNA bend
(D) It possesses intrinsic 3'-to-5' helicase activity

2011

**33.** The mediator complex is best described as a multi-protein bridge that connects which two components?
(A) RNA Polymerase I and ribosomal RNA sequences
(B) General factors and specific activators
(C) DNA polymerase and the replication helicase
(D) The spliceosome and the 5' end of mRNA

2015

**34.** Following the binding of TFIID, which factor is typically recruited next to stabilize the TBP-DNA interaction and assist in recruiting RNA Polymerase II?
(A) TFIIE
(B) TFIIH
(C) TFIIF
(D) TFIIB

2024

**35.** The phosphorylation of the Serine 5 residues on the CTD of RNA Polymerase II by TFIIH directly causes the polymerase to undergo which transition?
(A) Promoter clearance and elongation
(B) Specific promoter binding
(C) Pre-initiation complex disassembly
(D) Transcription termination

2011

**36.** Identify the INCORRECT statement regarding the eukaryotic pre-initiation complex.
(A) TFIID contains TBP and multiple TBP-associated factors (TAFs)
(B) TFIIH exhibits both kinase and helicase activities
(C) The mediator complex binds directly to the TATA box to initiate transcription
(D) TFIIF binds RNA polymerase II and helps bring it to the promoter

2002

**37.** In comparing prokaryotic and eukaryotic transcription initiation, what serves a functionally analogous role to the bacterial sigma factor?
(A) The general transcription factors (e.g., TFIID)
(B) The mediator complex
(C) RNA Polymerase II core enzyme
(D) Enhancers

2022

**38.** Identify the correct pair regarding general transcription factors and their roles.
(A) TFIIH : binds the TATA box directly
(B) TBP : RNA helicase activity
(C) TFIIA : phosphorylates the CTD
(D) TFIIE : recruits and stimulates TFIIH

2019

**39.** Which general transcription factor arrives at the promoter pre-bound to RNA Polymerase II?
(A) TFIID
(B) TFIIF
(C) TFIIH
(D) TFIIB

2012

**40.** Consider the following statements about eukaryotic transcription initiation:
I. The CTD of RNA Polymerase II contains heptapeptide repeats.
II. TFIIH unwinds the DNA at the transcription start site.
III. TBP bends the DNA by approximately 80 degrees.
IV. TFIIA acts as the primary kinase for CTD phosphorylation.

Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II and III

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**240.** (A) III → I → II → IV → V
The process of specialized transduction begins with the infection of a host cell and the precise integration of viral DNA. Later, induction of the lytic cycle occurs, sometimes with improper excision including host genes. These hybrid molecules are packaged and released upon cell lysis. Finally, the resulting transducing phage infects a recipient cell, delivering the donor DNA which can then integrate.

**241.** (A) Wild-type allele
Seymour Benzer demonstrated that recombination can occur at any point within a gene, even between mutations located close together in the same cistron. When two phages with different mutant alleles are in a trans configuration, a crossing over event between the two mutation sites can occur. This recombination can result in a DNA molecule that contains neither mutation, reconstructing a wild-type.

**242.** (C) Closely linked on the bacterial chromosome
Mapping the bacterial chromosome often utilizes generalized transduction. Because a bacteriophage capsid can only hold a small, fixed amount of DNA, there is a physical limit to the bacterial fragment size transferred. Consequently, two genes will only be co-transduced if they are located very close together. The frequency of co-transduction is therefore a direct measure of physical genetic linkage.

**243.** (A) Mitotic crossing over followed by haploidization
In many fungi lacking a traditional sexual lifecycle, the parasexual cycle provides an alternative means of generating genetic diversity. This cycle does not involve meiosis. Instead, it relies on infrequent mitotic crossing over to reshuffle alleles. This is followed by haploidization, a process of gradual and random chromosome loss that restores the haploid state. This cycle produces recombinant progeny in asexual species.

**244.** (B) Second division segregation
Tetrad analysis in linear fungi like Neurospora involves examining spore arrangements. A 2:4:2 or 2:2:2:2 arrangement of alleles is indicative of second division segregation. This pattern occurs when a crossing over event takes place between the gene locus and the centromere during the first meiotic division. Because of this recombination, the two different alleles are not separated until the second division.

**245.** (A) 1:1
In the study of fungal genetics through tetrad analysis, the relationship between different ditypes provides information about gene linkage. For two genes located on different chromosomes, the orientation of homologous pairs during meiosis is random. Statistically, this means that the formation of Parental Ditypes and Non-Parental Ditypes is equally likely. Analysis of many asci will yield a ratio of 1:1.

**246.** (C) Class II DNA transposons
Retroviruses and certain transposable elements share a similar strategy for integrating genetic material into a host genome. The enzyme retroviral integrase is responsible for the permanent insertion of the viral DNA copy. This enzyme functions in a manner that is fundamentally similar to the transposase found in Class II DNA transposons. Both enzymes recognize specific sequences and catalyze rejoining reactions for integration.

NODIA

**247.** (D) A functional transposase enzyme
In the maize genetic system described by McClintock, the mobility of the Ds element is dependent on the presence of the Ac element. Ac is an autonomous element, meaning it contains a functional gene encoding the transposase enzyme. The Ds element, a mutated version of Ac, lacks a functional transposase. If an Ac element is present, its transposase can recognize and move Ds.

**248.** (A) Retroviruses
Class I transposable elements, or retrotransposons, are a major component of eukaryotic genomes. These elements move through an RNA intermediate converted back into DNA by reverse transcriptase. This "copy-and-paste" mechanism is virtually identical to the replication cycle of retroviruses. Because of these functional similarities, it is widely believed that retrotransposons and retroviruses share a common evolutionary origin and molecular machinery.

**249.** (B) Chromosome breakage and mutable phenotypes
The movement of transposable elements can have profound effects on genomic structure.

first modification is the addition of a 5' cap, occurring almost immediately after transcription begins. This is followed by the removal of introns through splicing. Finally, the 3' end is cleaved and a poly-A tail is added. This specific maturation sequence is essential.

**231.** (D) Tetramer
The lac repressor is the key regulatory protein of the lac operon in Escherichia coli. In its fully functional state, the repressor exists as a homotetramer, formed by four identical polypeptide chains. Each dimer within the tetramer can bind to one operator site. This multimeric structure allows the repressor to bind to two operator sequences simultaneously, causing DNA looping and silencing.

**232.** (A) cAMP
Catabolite repression is a regulatory mechanism that allows bacteria to prioritize the use of glucose. When glucose is abundant, cellular cyclic AMP levels are low. However, when glucose is depleted, the activity of adenylate cyclase increases, leading to a rise in intracellular cAMP concentrations. cAMP then acts as a secondary messenger by binding to the catabolite activator protein to stimulate transcription.

**233.** (B) Transversion
A nonsense mutation is a genetic change that converts an amino acid codon into a premature stop signal. For example, if a tyrosine codon (UAC) is mutated to a stop codon (UAA), translation will end prematurely. This specific change involves replacing a cytosine (pyrimidine) with an adenine (purine). This replacement between different nitrogenous base classes is classified as a transversion mutation.

**234.** (A) Uracil
Nitrous acid is a potent chemical mutagen that induces mutations by causing the oxidative deamination of nitrogenous bases. It primarily targets bases containing amino groups, such as cytosine. When nitrous acid reacts with cytosine, the amino group is removed and replaced with a keto group, converting the base into uracil. Because uracil pairs with adenine, this modification leads to transition mutations.

**235.** (A) 3' to 5' exonuclease (proofreading) activity
Taq DNA polymerase is a thermostable enzyme widely used in PCR. However, it has a relatively high error rate because it intrinsically lacks a 3' to 5' exonuclease activity, which is responsible for proofreading. Without this ability to recognize and remove incorrectly incorporated nucleotides, the enzyme cannot correct its mistakes. Consequently, unintended mutations can be introduced during the DNA amplification.

**236.** (C) Centrally located within the primer sequence
When performing PCR-based site-directed mutagenesis to create a single amino acid substitution, the design of the mutagenic primer is critical. To ensure efficient binding, the primer should be mostly complementary to the template. The specific nucleotide mismatch should be centrally located within the primer sequence. This placement provides enough stable, perfectly matched base pairs on either side to allow for specific annealing.

**237.** (D) Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
The mismatch repair system is essential for maintaining genomic stability by correcting errors that escape the proofreading activity of DNA polymerases. In humans, mutations in the genes that encode core proteins of the MMR pathway, such as MSH2, lead to high genetic instability. This condition is the primary cause of Hereditary Non-Polyposis Colorectal Cancer, where patients have increased cancer risks.

**238.** (D) Xeroderma pigmentosum
Nucleotide excision repair is a vital pathway for removing bulky DNA lesions, particularly those induced by exposure to ultraviolet radiation. In humans, this process involves a complex of many proteins. Genetic defects in any of the genes encoding these repair proteins result in a severe condition known as Xeroderma pigmentosum. Individuals with this disease are extremely sensitive to sunlight and skin cancers.

**239.** (D) The exact site of prophage integration in the bacterial chromosome
Specialized transduction is a highly specific mechanism of horizontal gene transfer that occurs only in temperate bacteriophages. The process is entirely dependent on the fact that these phages integrate their DNA into the host chromosome at a precise, fixed location. Because the phage integrates at the same spot, an improper excision event will always capture the same neighboring host genes for transfer.

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# CHAPTER 05
# Gene Expression

2006

**1.** Which sequence element is primarily responsible for directing the accurate initiation of transcription by defining the precise transcription start site (+1) in eukaryotes?
(A) TATA box
(B) Initiator element
(C) GC box
(D) CAAT box

2013

**2.** What is the consensus sequence of the Pribnow box found in prokaryotic promoters?
(A) TTGACA
(B) TATAAA
(C) CAAT
(D) TATAAT

2015

**3.** Which statement accurately describes the location of the -35 sequence in a typical prokaryotic promoter?
(A) It is centered approximately 35 base pairs upstream of the transcription start site
(B) It is located 35 base pairs downstream of the transcription start site
(C) It is located 35 base pairs upstream of the Pribnow box
(D) It serves as the transcription start site at position +35

2007

**4.** Based on transcription unit architecture, which element is characteristically found in eukaryotic promoters but absent in prokaryotic promoters?
(A) Pribnow box
(B) -35 sequence
(C) GC box
(D) Coding sequence

2002

**5.** The primary cause of transcription initiation accurately starting at the +1 site in prokaryotes is the binding of the sigma factor to which specific region?
(A) Both the -10 and -35 sequences
(B) The primary gene coding sequence
(C) The eukaryotic initiator element
(D) The proximal CAAT box sequence

2001

**6.** Identify the INCORRECT statement regarding eukaryotic promoter elements.
(A) The TATA box is usually located around position -25 to -30
(B) The CAAT box frequently operates to determine the efficiency of the promoter
(C) GC boxes are typically recognized by the Sp1 transcription factor
(D) All eukaryotic promoters require a TATA box for transcription initiation

2004

**7.** Compared to the core promoter, what is the primary role of the proximal promoter elements such as the CAAT box?
(A) To dictate the exact base where transcription begins
(B) To modulate the frequency of transcription initiation
(C) To act as a binding site for general transcription factors
(D) To terminate transcription

NODIA

2007

**8.** Identify the correct pair regarding promoter elements and their common position relative to the transcription start site.
(A) Pribnow box : specific to the -35 region
(B) TATA box : at position -10 in eukaryotes
(C) -35 sequence : prokaryotic core promoter
(D) Initiator element : between -50 and -100

2005

**9.** Which element is generally recognized first by TFIID during eukaryotic transcription initiation?
(A) GC box
(B) Pribnow box
(C) -35 sequence
(D) TATA box

2007

**10.** The following question consists of two statements—Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.

When an element integrates into a new site, it can disrupt functional genes, leading to mutations. Furthermore, the excision of elements, particularly in maize systems, can cause chromosomal breakage and instability. These events often result in variegated or mutable phenotypes, where the physical appearance of the organism changes during development.

**250.** (B) Encoding only the proteins required for their own transposition
Insertion Sequences are the simplest transposable elements found in prokaryotic organisms. Their genetic role is strictly limited to their own mobilization. An IS element typically contains only a single gene, which encodes the transposase enzyme, flanked by inverted repeats. Unlike complex transposons, IS elements do not carry any additional genes. Their primary impact is the potential to cause mutations or rearrangements.

**********

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List II: (Sensitivity to α-amanitin)
1. Highly sensitive
2. Moderately sensitive (species dependent)
3. Insensitive
4. Insensitive (bacterial)

(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-3, R-4, S-1

2012

**21.** Which mechanism characterizes intrinsic (rho-independent) termination of transcription in prokaryotes?
(A) GC-rich hairpin loop followed by a run of U residues
(B) Binding of the specific rho protein to the rut site
(C) Phosphorylation of the polymerase C-terminal domain
(D) Cleavage of the nascent transcript by an endonuclease

2009

**22.** In rho-dependent termination, what specific enzymatic activity does the rho protein utilize to dissociate the RNA-DNA hybrid?
(A) Sequence-specific RNAse activity
(B) Standard DNA ligase activity
(C) Peptidyl transferase activity
(D) ATP-dependent RNA-DNA helicase

2025

**23.** During prokaryotic transcription elongation, the formation of phosphodiester bonds is coupled with the release of which byproduct?
(A) ATP
(B) Pyrophosphate
(C) Cyclic AMP
(D) Inorganic phosphate

2001

**24.** A mutation in the rut site of a prokaryotic gene will most directly affect which process?
(A) Promoter recognition by sigma factor
(B) Intrinsic termination
(C) Rho-dependent termination
(D) Transcription initiation

2008

**25.** The presence of a poly-U sequence at the 3' end of a prokaryotic transcript directly causes which event during transcription?
(A) Stabilization of the internal RNA-DNA hybrid
(B) Direct recruitment of the rho factor helicase
(C) Immediate binding of the 30S ribosomal subunit
(D) RNA-DNA hybrid destabilization and detachment

2011

**26.** Identify the INCORRECT statement regarding prokaryotic transcription.
(A) Intrinsic termination requires ATP hydrolysis by a termination factor
(B) Transcription and translation can occur simultaneously
(C) The transcription bubble contains an RNA-DNA hybrid of about 8 to 9 base pairs
(D) The core polymerase maintains processivity during elongation

2016

**27.** Comparing transcription initiation and elongation in prokaryotes, which event marks the transition from initiation to elongation?
(A) The binding of the core enzyme to the promoter
(B) The release of the sigma factor and clearance of the promoter
(C) The synthesis of the first phosphodiester bond
(D) The formation of a hairpin loop

2005

**28.** Identify the correct pair regarding transcription phases.
(A) Initiation : processive addition of nucleotides
(B) Termination : dissociation of RNA polymerase from the DNA template
(C) Elongation : synthesis of short abortive transcripts
(D) Promoter clearance : binding of the sigma factor

2009

**29.** Which base pairing interaction is the most stable and heavily contributes to the structure of the termination hairpin in intrinsic termination?
(A) G-C
(B) A-U
(C) A-T
(D) C-U

2002

**30.** Consider the following statements about transcription termination in prokaryotes:
I. Rho-dependent termination requires the presence of a rut site on the nascent RNA.
II. Intrinsic termination requires a GC-rich inverted repeat.

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Assertion (A): The prokaryotic RNA polymerase holoenzyme can specifically recognize promoter sequences, whereas the core enzyme cannot.
Reason (R): The core enzyme lacks the sigma factor, which is required for sequence-specific binding to the -10 and -35 promoter elements.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**11.** Which subunit of the prokaryotic RNA polymerase core enzyme is responsible for the catalytic synthesis of RNA?
(A) A
(B) B
(C) Omega
(D) Sigma

2006

**12.** What distinguishes eukaryotic RNA polymerase I from RNA polymerase II?
(A) Polymerase I synthesizes mRNA, while polymerase II synthesizes rRNA
(B) Polymerase I synthesizes rRNA, while polymerase II synthesizes mRNA
(C) Polymerase I requires a sigma factor, while polymerase II does not
(D) Polymerase I is highly sensitive to α-amanitin, while polymerase II is resistant

2008

**13.** Which plant-specific RNA polymerase is primarily involved in RNA-directed DNA methylation and transcriptional gene silencing?
(A) RNA Polymerase IV
(B) RNA Polymerase I
(C) RNA Polymerase III
(D) RNA Polymerase II

2023

**14.** An experiment demonstrates that a specific RNA polymerase is inhibited by low concentrations of α-amanitin (1μg/ml). Which enzyme is most likely being tested?
(A) Prokaryotic RNA polymerase
(B) Eukaryotic RNA polymerase I
(C) Eukaryotic RNA polymerase II
(D) Eukaryotic RNA polymerase III

2005

**15.** The binding of a specific sigma factor directly causes the RNA polymerase holoenzyme to shift its preference. What is the effect of sigma-32 (RpoH) in E. coli?
(A) It directs the transcription of nitrogen metabolism genes
(B) It directs the transcription of bacterial flagellar genes
(C) It represses all cellular transcription during heat stress
(D) It directs transcription of heat shock protein genes

NODIA

2008

**16.** Identify the INCORRECT statement regarding the subunit composition of RNA polymerases.
(A) The prokaryotic core enzyme consists of $\alpha_2$, β, β', and omega subunits
(B) Eukaryotic RNA polymerases contain 10 to 14 subunits
(C) The omega subunit in prokaryotes is essential for the catalytic addition of nucleotides
(D) Eukaryotic RNA polymerases share some common subunits among Pol I, II, and III

2006

**17.** In a comparison of RNA polymerases, which eukaryotic polymerase is responsible for synthesizing tRNA and 5S rRNA?
(A) RNA Polymerase I
(B) RNA Polymerase III
(C) RNA Polymerase II
(D) RNA Polymerase V

2004

**18.** Identify the correct pair connecting an RNA polymerase with its primary transcript.
(A) RNA Polymerase I : 5S rRNA synthesis
(B) RNA Polymerase III : 28S rRNA synthesis
(C) Plant RNA Pol V : primary rRNA synthesis
(D) RNA Polymerase II : snRNA and mRNA

2021

**19.** What is the primary function of the α subunit in the prokaryotic RNA polymerase core enzyme?
(A) Enzyme assembly and binding to UP elements
(B) Catalytic synthesis of the RNA chain
(C) Unwinding the DNA double helix
(D) Recognizing the -10 sequence

2025

**20.** Match the items in List I with those in List II.
List I: (RNA Polymerase)
P. RNA Pol I
Q. RNA Pol II
R. RNA Pol III
S. Prokaryotic RNA Pol

(C) I and IV
(D) IV only

2011

**41.** By definition, what is a genetic enhancer?
(A) A sequence encoding a specific transcription factor
(B) A protein that binds directly to the promoter site
(C) A 5' UTR sequence that initiates protein translation
(D) A cis-acting sequence regulating distal transcription

2022

**42.** Which structural model explains how enhancers can regulate promoters located thousands of base pairs away?
(A) The DNA looping model
(B) The sliding clamp model
(C) The transcription attenuation model
(D) The riboswitch model

2010

**43.** The primary function of a genetic insulator is to:
(A) Increase the rate of protein translation
(B) Block enhancers from acting on distal promoters
(C) Facilitate nuclear RNA splicing
(D) Bind RNA polymerase to initiate transcription

2006

**44.** An enhancer sequence is flipped 180 degrees relative to its original orientation. What is the most likely functional consequence on its target promoter?
(A) Transcription is completely abolished
(B) Transcription remains elevated due to orientation-independence
(C) The enhancer now acts as a transcriptional silencer
(D) Transcription initiates in the reverse direction

2025

**45.** The binding of repressor proteins to a silencer element typically results in the recruitment of which type of enzymes to cause gene silencing?
(A) Histone deacetylases (HDACs)
(B) Histone acetyltransferases (HATs)
(C) RNA helicases
(D) DNA polymerases

2013

**46.** Identify the INCORRECT statement regarding insulators.
(A) They can block enhancer-promoter interactions if positioned between them
(B) They can act as barriers against the spread of heterochromatin
(C) Their activity is solely dependent on being located precisely at the -35 position
(D) CTCF is a well-known protein that binds to mammalian insulators

2023

**47.** In comparing enhancers and promoters, which characteristic is strictly true for promoters but not for enhancers?
(A) They bind transcription factors
(B) They can function downstream of the coding region
(C) They are composed of specific DNA sequences
(D) They dictate the precise start point and direction of transcription

2004

**48.** Identify the correct pair involving regulatory elements.
(A) Enhancer : orientation-dependent activity
(B) Silencer : recruits co-activators
(C) Insulator : restricts the action of enhancers
(D) Promoter : functions optimally when positioned 50 kb downstream

2010

**49.** Which phenomenon best illustrates the mechanism of long-distance gene regulation by enhancers?
(A) Alternative splicing
(B) Formation of a loop connecting the enhancer-bound activator to the mediator complex at the promoter
(C) Linear tracking of RNA polymerase from the enhancer to the promoter
(D) Release of the sigma factor

2018

**50.** Consider the following statements about eukaryotic regulatory elements:
I. Enhancers can function whether located upstream or downstream of a gene.
II. Silencers typically bind activator proteins to increase transcription.
III. Insulators can protect genes from positional effects in transgenic organisms.
IV. DNA looping is facilitated by structural proteins like cohesin.

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V. Repressors bound to silencers act by directly degrading RNA Polymerase II molecules.

Which of the above statement(s) is/are INCORRECT?
(A) II and V
(B) I, II and IV
(C) III and V
(D) I, III and IV

2010

**51.** By definition, what role does a co-activator play in transcription?
(A) It bridges activators and basal machinery
(B) It binds directly to DNA promoter sequences
(C) It cleaves the newly synthesized RNA
(D) It methylates the DNA to repress transcription

2003

**52.** The domain structure of a typical transcriptional activator consists of:
(A) A domain that binds DNA and another domain for protein recruitment
(B) Only an enzymatic domain for RNA synthesis
(C) A DNA-binding domain and an activation domain
(D) Only a nuclear export signal

2012

**53.** CBP/p300 is an important transcriptional co-activator. What intrinsic enzymatic activity does it possess that facilitates transcription?
(A) Histone acetyltransferase (HAT) activity
(B) Functional DNA ligase activity
(C) Histone deacetylase (HDAC) activity
(D) Genomic RNA polymerase activity

2008

**54.** A researcher treats cells with an inhibitor of histone deacetylases (HDACs). What is the expected general effect on gene expression?
(A) Global transcriptional repression
(B) Immediate destruction of all RNA transcripts
(C) Prevention of mRNA export
(D) Global transcriptional activation

2002

**55.** The recruitment of histone deacetylases (HDACs) by a co-repressor directly causes which change in chromatin structure?
(A) Addition of acetyl groups to DNA
(B) Acetyl group removal and chromatin compaction
(C) Acetyl group addition and chromatin opening
(D) Complete dissociation of histones from DNA

2013

**56.** Identify the INCORRECT statement regarding specific transcription regulators.
(A) Activators can recruit chromatin remodeling complexes
(B) Repressors can compete with activators for the same DNA binding sites
(C) Co-activators must possess a specific DNA-binding domain to function
(D) Activation domains often contain high proportions of acidic, glutamine-rich, or proline-rich sequences

2003

**57.** Comparing activators and co-activators, what is the primary operational distinction between them?
(A) Co-activators bind specific DNA sequences; activators do not
(B) Activators exhibit HAT activity; co-activators never do
(C) Activators function only in prokaryotes; co-activators only in eukaryotes
(D) Activators bind DNA; co-activators bridge proteins

2024

**58.** Identify the correct pair involving a transcriptional regulator and its typical function.
(A) Activation domain : Interacts with the basal transcription machinery
(B) CBP/p300 : Co-repressor
(C) Histone deacetylase : Co-activator
(D) DNA-binding domain : Possesses histone kinase activity

NODIA

2009

**59.** Which structural mechanism allows a single repressor protein to inhibit multiple different genes?
(A) By directly digesting the mRNA of target genes
(B) By interacting with a common co-repressor complex
(C) By converting the target DNA into antisense RNA
(D) By binding to the telomeres of the chromosome

2014

**60.** The following question consists of two statements—Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.

Assertion (A): Histone acetylation by CBP/p300 generally correlates with transcriptional activation.
Reason (R): The addition of negatively charged acetyl groups neutralizes the positive charge of lysine residues on histones, decreasing their affinity for DNA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2013

**61.** The structural feature of the basic Leucine Zipper (bZIP) motif that facilitates dimerization is:
(A) An amphipathic α-helix with periodic leucines
(B) A β-sheet containing multiple leucine residues
(C) A single zinc ion coordinated by four leucine residues
(D) A loop region rich in leucine and proline

2001

**62.** Which class of zinc finger motif coordinates a single zinc ion utilizing two cysteines and two histidines?
(A) C4
(B) bHLH
(C) $C_2H_2$
(D) bZIP

2010

**63.** In the Helix-turn-helix (HTH) motif, what is the primary role of the "recognition helix"?
(A) It binds to the minor groove of the DNA
(B) It forms a dimer with another HTH motif
(C) It coordinates a zinc ion
(D) It recognizes specific base pairs in the major groove

2005

**64.** A transcription factor contains a basic Helix-Loop-Helix (bHLH) motif. What function does the highly basic region adjacent to the HLH domain serve?
(A) Protein dimerization
(B) Nuclear localization
(C) DNA binding
(D) Ligand binding

2011

**65.** The substitution of a critical histidine residue in a $C_2H_2$ zinc finger motif with an alanine would most directly cause:
(A) Enhanced dimerization capability
(B) Disrupted zinc coordination and DNA binding loss
(C) Conversion of the motif into a leucine zipper
(D) Shift in binding from the major groove to the minor groove

2012

**66.** Identify the INCORRECT statement regarding DNA-binding motifs.
(A) Most transcription factors recognize sequences in the major groove
(B) The C4 zinc finger is commonly found in steroid hormone receptors
(C) The helix-turn-helix motif lacks a zinc ion for its structural stability
(D) Leucine zippers bind DNA directly using the leucine-rich dimerization helix

2012

**67.** Compared to the $C_2H_2$ zinc finger, the C4 zinc finger motif differs primarily in that it:
(A) Coordinates zinc via four cysteines and mediates dimerization
(B) Coordinates zinc via four histidines
(C) Functions exclusively as a monomer
(D) Coordinates iron instead of zinc

2015

**68.** Identify the correct pair involving a DNA-binding motif and its characteristic feature.
(A) Leucine zipper : Coordinates zinc via leucine residues
(B) Helix-turn-helix : Forms an amphipathic dimerization interface
(C) $C_2H_2$ zinc finger : Found as tandem repeats inserting α helices into the major groove
(D) bHLH : Exclusively binds RNA

2006

**69.** Why do most sequence-specific DNA-binding motifs insert an α-helix into the major groove rather than the minor groove?
(A) The major groove has more unique recognition patterns
(B) The major groove is too narrow to accommodate β-sheets
(C) The minor groove lacks hydrogen bond donors and acceptors
(D) The minor groove is blocked by histones

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by negative feedback.
II. The apo-repressor cannot bind the operator without tryptophan.
III. Attenuation provides a second layer of regulation sensitive to tRNA charging levels.
IV. The formation of the 1:2 hairpin is essential for antitermination.

Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I, II and III
(D) I and III only

2014

**91.** The 5' cap added to eukaryotic pre-mRNA consists primarily of which modified nucleotide?
(A) 7-methyladenosine
(B) 7-methylguanosine
(C) N6-methyladenosine
(D) 5-methylcytidine

2024

**92.** The linkage connecting the 5' cap to the first nucleotide of the mRNA transcript is unique. What type of linkage is it?
(A) 3'-to-5' phosphodiester bond
(B) 5'-to-5' triphosphate bridge
(C) 2'-to-5' phosphodiester bond
(D) Peptide bond

2013

**93.** In the enzymatic pathway of capping, which enzyme is responsible for removing the terminal (gamma) phosphate from the 5' end of the nascent transcript?
(A) RNA triphosphatase
(B) Guanylyltransferase
(C) Guanine-N7-methyltransferase
(D) Poly(A) polymerase

2006

**94.** A failure in the function of guanylyltransferase will directly cause which defect in eukaryotic mRNA?
(A) Failure to methylate an existing cap
(B) Absence of a poly(A) tail
(C) Complete absence of the 5' cap structure
(D) Inhibition of RNA splicing

2008

**95.** The presence of the 5' cap structure directly causes the recruitment of which initiation factor during translation?
(A) eIF2
(B) eIF3
(C) $IF_1$
(D) The cap-binding protein eIF4E

2003

**96.** Identify the INCORRECT statement regarding eukaryotic mRNA cap structure variants.
(A) Cap 0 has only the 7-methylguanosine added without further methylation of the transcript's ribose sugars
(B) Cap 1 involves methylation of the 2'-O position of the first transcribed nucleotide
(C) Cap structures are added post-translationally in the cytoplasm
(D) Cap 2 involves methylation of the 2'-O position of both the first and second transcribed nucleotides

2004

**97.** Comparing bacterial and eukaryotic mRNA, what is a primary functional reason eukaryotes utilize a 5' cap while bacteria do not?
(A) Bacteria lack ribosomes
(B) The cap enables nuclear export and ribosome scanning
(C) Bacterial mRNAs are translated inside the nucleus
(D) Bacterial mRNAs are exceptionally stable and do not degrade

2011

**98.** Identify the correct pair involving capping enzymes and their actions.
(A) RNA triphosphatase : adds a methyl group to guanine
(B) Methyltransferase : removes the gamma phosphate
(C) Guanylyltransferase : operates at the 3' end of the mRNA
(D) Guanylyltransferase : transfers GMP to the 5' end

2009

**99.** Which portion of the RNA polymerase II transcription machinery serves as a scaffold to recruit the capping enzymes shortly after initiation?
(A) The phosphorylated C-terminal domain
(B) The TATA-binding protein complex
(C) The multi-subunit mediator complex
(D) The zinc finger DNA-binding motif

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2008

**100.** Consider the following statements regarding mRNA capping:
I. The cap protects mRNA from 5' to 3' exonucleases.
II. Capping occurs only after the entire

that prevents repressor binding results in constitutive expression.

III. lacA encodes a permease that transports lactose.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I only

2011

**81.** By definition, a repressible operon like the trp operon is characterized by being:

(A) Normally off, but turned on by an inducer
(B) Normally on, but turned off by the presence of a co-repressor
(C) Permanently active regardless of environmental conditions
(D) Regulated only by positive control mechanisms

2011

**82.** In the regulation of the trp operon, what molecule acts as the co-repressor?

(A) cAMP
(B) Glucose
(C) The Trp repressor protein
(D) Tryptophan

2017

**83.** The leader peptide sequence (trpL) of the trp operon features tandem consecutive codons for which specific amino acid?

(A) Tryptophan
(B) Methionine
(C) Proline
(D) Arginine

2006

**84.** A mutation eliminates the two adjacent tryptophan codons in the leader sequence. How will this affect transcription attenuation in the trp operon?

(A) The operon will be fully transcribed regardless of tryptophan levels
(B) The ribosome will stall permanently, blocking all transcription
(C) Ribosome stalling is lost, causing premature attenuation
(D) The repressor will bind irreversibly to the operator

2017

**85.** During high intracellular tryptophan levels, the ribosome rapidly translates the leader peptide. This rapid movement directly causes the RNA to form which structure?

(A) The 3:4 terminator hairpin
(B) The 2:3 antiterminator hairpin
(C) A circular RNA
(D) A lariat structure

2023

**86.** Identify the INCORRECT statement regarding trp operon attenuation.

(A) Attenuation relies on the tight coupling of transcription and translation in prokaryotes
(B) The formation of the 2:3 hairpin prevents the formation of the 3:4 hairpin
(C) Low levels of charged tRNA-Trp cause the ribosome to stall at region 1
(D) Attenuation is a mechanism of positive control that requires cAMP

2012

**87.** Comparing the lac and trp operons, which feature is unique to the trp operon's regulatory repertoire?

(A) Regulation by a repressor protein
(B) Ribosome-mediated transcription attenuation
(C) The presence of an operator region
(D) Multiple structural genes transcribed as a single polycistronic mRNA

2022

**88.** Identify the correct pair involving the trp operon.

(A) Trp repressor : binds operator in the absence of tryptophan
(B) Attenuation : terminates translation but not transcription
(C) Antiterminator loop : formed by base pairing between regions 2 and 3
(D) Co-repressor : allolactose

2015

**89.** What is the fundamental basis for the ribosome stalling during trp operon attenuation?

(A) A physical block caused by the RNA polymerase
(B) The binding of a release factor to the tandem codons
(C) A strong stop codon immediately following the start codon
(D) Depletion of charged tryptophanyl-tRNA during starvation

2019

**90.** Consider the following statements about the trp operon:

I. It is a repressible operon primarily regulated

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Answer the question by selecting the appropriate option.
Assertion (A): Polyadenylation of eukaryotic mRNA is an essential step for its successful translation.
Reason (R): The poly(A) tail is required to assemble the spliceosome and remove introns from the pre-mRNA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2006

**111.** The core components of the major spliceosome are primarily composed of:
(A) Small nucleolar RNAs (snoRNAs) and proteins
(B) Ribosomal RNAs (rRNAs)
(C) Transfer RNAs (tRNAs)
(D) Small nuclear ribonucleoproteins (snRNPs)

2003

**112.** Which specific chemical reaction drives the splicing of nuclear pre-mRNA?
(A) Hydrolysis
(B) Dehydration synthesis
(C) Transesterification
(D) Oxidative phosphorylation

2002

**113.** What is the fundamental structure of the intron intermediate formed after the first catalytic step of splicing?
(A) A linear piece of RNA
(B) Lariat structure with 2'-5' phosphodiester bond
(C) A circular piece of RNA
(D) A double-stranded RNA helix

2007

**114.** A mutation at the highly conserved invariant 'A' residue at the branch point sequence will most likely cause:
(A) Premature polyadenylation
(B) Accelerated splicing kinetics
(C) Exon skipping
(D) Failure of the first step, preventing lariat formation

2002

**115.** During spliceosome assembly, the binding of U1 snRNP to the 5' splice site directly causes the recruitment of which snRNP to the branch point?
(A) U2
(B) U4
(C) U5
(D) U6

2018

**116.** Identify the INCORRECT statement regarding nuclear pre-mRNA splicing.
(A) U4/U6 di-snRNP complex arrives as part of a tri-snRNP with U5
(B) U1 and U4 snRNPs must leave the complex before the first catalytic step can occur
(C) The first transesterification involves the 3' OH of the upstream exon attacking the 5' splice site
(D) U6 is the primary catalytic RNA of the spliceosome

2023

**117.** Comparing the two transesterification steps of splicing, what attacks the 3' splice site in the second step?
(A) The 3' OH of the free 5' exon
(B) The 2' OH of the branch point adenosine
(C) The 5' phosphate of the intron
(D) The 2' OH of the free 5' exon

2015

**118.** Identify the correct pair involving snRNPs and their functions.
(A) U1 snRNP : binds to the 3' splice site
(B) U5 snRNP : catalyzes lariat formation
(C) U4 snRNP : directly attacks the 5' splice site
(D) U2 snRNP : binds to the branch point sequence

2004

**119.** Alternative splicing represents a crucial regulatory mechanism. What is its primary advantage to a eukaryotic organism?
(A) It completely eliminates the need for transcriptional regulation
(B) Single genes encoding multiple protein isoforms
(C) It increases the speed of translation
(D) It repairs mutations in the genomic DNA

2011

**120.** Match the items in List I with those in List II.
List I: (Spliceosome component or site)
P. 5' splice site
Q. 3' splice site
R. Branch point
S. U6 snRNA

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transcript has been fully synthesized.
III. S-adenosylmethionine (SAM) serves as the methyl donor for cap methylation.
IV. The cap structure is necessary for efficient pre-mRNA splicing of the first intron.
V. Plant mitochondria use a 5' cap for all of their mRNAs.

Which of the above statement(s) is/are INCORRECT?
(A) II and V
(B) I, III and IV
(C) III and IV
(D) I and II

2006

**101.** Which specific consensus sequence primarily directs the cleavage and polyadenylation of eukaryotic pre-mRNA?
(A) AAUAAA
(B) TATAAA
(C) GGCCAATCT
(D) Shine-Dalgarno sequence

2022

**102.** What is the role of Poly(A) Polymerase (PAP) in the processing of eukaryotic mRNA?
(A) It synthesizes the poly(A) tail using an RNA template
(B) It cleaves the pre-mRNA at the polyadenylation site
(C) It methylates the 3' end of the mRNA
(D) It adds adenines in a template-independent manner

2011

**103.** Which factor physically recognizes and binds to the AAUAAA signal sequence during the 3' processing of pre-mRNA?
(A) Cleavage stimulation factor (CstF)
(B) The CPSF specificity factor
(C) Poly(A) binding protein (PABP)
(D) Cleavage factor I (CFI)

2017

**104.** A mutation occurs in the G/U-rich element located downstream of the polyadenylation cleavage site. What is the most likely consequence?
(A) PAP synthesizes an exceptionally long poly(A) tail
(B) The mRNA immediately circularizes in the nucleus
(C) CstF binding fails, reducing cleavage efficiency
(D) The 5' cap is removed

2018

**105.** The addition of the poly(A) tail directly causes which outcome for the mature mRNA molecule?
(A) Inhibition of nuclear export
(B) Increased susceptibility to 3' exonucleases
(C) Immediate transport to the nucleolus
(D) Enhanced stability and translation initiation

2025

**106.** Identify the INCORRECT statement regarding 3' polyadenylation.
(A) Cleavage of the pre-mRNA occurs upstream of the AAUAAA sequence
(B) Histone mRNAs in metazoans typically lack a poly(A) tail
(C) Poly(A) polymerase requires a primer but no template
(D) Poly(A) binding proteins coat the tail to protect it from degradation

2012

**107.** Comparing capping and polyadenylation, which characteristic applies uniquely to polyadenylation?
(A) It occurs co-transcriptionally via the CTD of RNA Pol II
(B) It involves the addition of nucleotides without an RNA or DNA template
(C) It involves a 5'-to-5' triphosphate linkage
(D) It prevents degradation by exonucleases

2022

**108.** Identify the correct pair involving polyadenylation factors.
(A) CPSF : binds the downstream G/U-rich element
(B) PAP : requires ATP as a substrate
(C) CstF : binds the AAUAAA hexamer
(D) PABP : cleaves the transcript

2023

**109.** What physical interaction provides the link between the poly(A) tail and the 5' cap during translation initiation, effectively circularizing the mRNA?
(A) PABP interaction with the eIF4G subunit
(B) Interaction between CPSF and TFIID
(C) Direct base-pairing between the poly(A) tail and the cap
(D) Binding of the 40S ribosomal subunit to both ends simultaneously

2024

**110.** The following question consists of two statements—Assertion (A) and Reason (R).

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III. ADAR enzymes require double-stranded RNA structures to function.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2006

**131.** Which feature of the genetic code indicates that a single amino acid can be encoded by more than one codon?

(A) Degeneracy
(B) Universality
(C) Non-overlapping nature
(D) Lack of punctuation

2005

**132.** What constitutes a synonymous codon?

(A) A codon that pairs with multiple different tRNAs
(B) Codons that specify chemically similar amino acids
(C) Codons that specify the same amino acid
(D) Codons that signal translation termination

2022

**133.** According to the Wobble Hypothesis proposed by Francis Crick, which position of the codon is subject to relaxed base-pairing rules?

(A) The third position (3' end)
(B) The first position (5' end)
(C) The second position
(D) All three positions

2017

**134.** An mRNA sequence reads 5'-AUG-3'. The corresponding anticodon on the tRNA (read in the standard 5' to 3' direction) would be:

(A) 5'-UAC-3'
(B) 5'-GUA-3'
(C) 5'-AUG-3'
(D) 5'-CAU-3'

2014

**135.** The phenomenon of codon bias is best defined as:

(A) The exclusive use of one codon for a specific amino acid
(B) Non-random usage of synonymous codons by organisms
(C) The mutation of codons strictly at the third position
(D) The overlapping reading of codons in viruses

2003

**136.** Identify the INCORRECT statement regarding the genetic code.

(A) The code is read in a non-overlapping manner
(B) There are 61 sense codons and 3 stop codons in the standard code
(C) The code is universal, with no known exceptions in mitochondrial or viral genomes
(D) Degeneracy typically minimizes the deleterious effects of point mutations at the third codon position

2019

**137.** Comparing the standard nuclear genetic code and the mitochondrial genetic code, which variation is commonly observed in human mitochondria?

(A) AUG codes for Leucine instead of Methionine
(B) UAA codes for Tyrosine instead of a stop signal
(C) CUG codes for Serine instead of Leucine
(D) UGA codes for Tryptophan instead of a stop signal

2025

**138.** Identify the correct pair involving wobble base pairing between the 5' anticodon base and the 3' codon base.

(A) Inosine (I) : pairs with A, U, or C
(B) Guanine (G) : pairs only with C
(C) Uracil (U) : pairs with G, C, or A
(D) Cytosine (C) : pairs with G or U

2017

**139.** If a frameshift mutation inserts one nucleotide early in a coding sequence, what is the primary consequence?

(A) Only one amino acid is altered in the final protein
(B) Downstream frame alteration and premature termination
(C) The reading frame is immediately restored by wobble pairing
(D) Translation is not affected due to degeneracy

2024

**140.** Consider the following statements about the genetic code:

I. It is a triplet code containing 64 possible codons.
II. Inosine in the first position of the anticodon maximizes the wobble effect.
III. Mitochondria strictly use the universal genetic code without any deviations.
IV. Synonymous mutations do not change the amino acid sequence of the protein.

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List II: (Specific feature)
1. Usually features an AG dinucleotide
2. Contains an invariant Adenine required for lariat formation
3. Typically starts with a GU dinucleotide
4. Acts as the central catalytic component of the active spliceosome

(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-2, R-1, S-4
(D) P-4, Q-1, R-2, S-3

2004

**121.** What defines a self-splicing intron?
(A) An intron that relies on the host spliceosome for its own splicing
(B) An intron that is spliced out entirely by DNA polymerase
(C) An intron found only in prokaryotic mRNA
(D) RNA catalyzing its excision without a spliceosome

2021

**122.** Group I introns utilize an external cofactor to initiate splicing. Which molecule serves this role?
(A) A free guanosine nucleotide or nucleoside
(B) A free adenosine nucleotide
(C) ATP
(D) U1 snRNP

2014

**123.** Which catalytic mechanism characterizes Group II intron splicing?
(A) An external guanosine attack the 5' splice site
(B) An internal branch point adenosine uses its 2'-OH to attack the 5' splice site, forming a lariat
(C) Cleavage by an external protein endonuclease followed by ligation
(D) Hydrolysis of the phosphodiester bond by water

2017

**124.** The fundamental mechanism of cytidine-to-uridine (C-to-U) RNA editing involves which type of enzymatic reaction?
(A) Methylation
(B) Deamination
(C) Transamination
(D) Phosphorylation

2014

**125.** The conversion of an adenosine to inosine (A-to-I) in a coding sequence directly causes the translation machinery to read the inosine as which base?
(A) Guanine
(B) Adenine
(C) Cytosine
(D) Uracil

2004

**126.** Identify the INCORRECT statement regarding self-splicing introns.
(A) They are considered ribozymes
(B) Group I introns form a characteristic secondary structure with paired elements (P1-P9)
(C) Group I introns generate a lariat intermediate
(D) Group II splicing is mechanistically very similar to spliceosomal splicing

2004

**127.** Comparing Group I and Group II introns, what is a distinctive difference in their excised forms?
(A) Group I introns are excised as lariats, Group II as linear molecules
(B) Both are excised as linear molecules
(C) Both are excised as circles
(D) Group I introns are excised as linear molecules, Group II as lariats

2019

**128.** Identify the correct pair involving RNA editing.
(A) ADAR enzyme : catalyzes C-to-U deamination
(B) Trypanosome kinetoplasts : editing by deamination
(C) APOB gene : C-to-U editing creates a premature stop codon in the intestine
(D) A-to-I editing : typically occurs in unstructured single-stranded RNA

2022

**129.** How does ADAR-mediated A-to-I RNA editing affect an organism?
(A) It strictly functions to degrade viral RNA
(B) Altered protein sequence affecting neuroreceptors
(C) It removes introns from the RNA
(D) It adds a poly(A) tail to the edited transcript

2011

**130.** Consider the following statements about RNA processing:
I. Group I introns require an external guanosine for the first step of splicing.
II. RNA editing by guide RNAs (gRNAs) primarily involves insertion or deletion of uridine residues.

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2005

**70.** Match the items in List I with those in List II.
List I: (DNA-binding motif)
P. Helix-turn-helix
Q. Leucine zipper
R. $C_2H_2$ Zinc finger
S. Basic Helix-loop-helix
List II: (Structural feature)
1. Amphipathic α helix with leucines every 7th position
2. Two cysteines and two histidines coordinating a metal ion
3. Two α helices connected by a short amino acid sequence, lacking metal ions
4. Two α helices separated by a flexible variable-length loop region
(A) P-1, Q-2, R-4, S-3
(B) P-3, Q-1, R-2, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-2, Q-4, R-3, S-1

2005

**71.** In the context of the lac operon, what defines the role of allolactose?
(A) It is the primary repressor protein
(B) It acts as an inducer by altering repressor conformation
(C) It is the promoter sequence
(D) It binds directly to the CAP protein

2007

**72.** Which gene of the lac operon encodes the enzyme β-galactosidase?
(A) lacY
(B) lacI
(C) lacZ
(D) lacA

2025

**73.** What is the fundamental mechanism of "inducer exclusion" in the regulation of the lac operon?
(A) High lactose levels prevent glucose from entering the cell
(B) cAMP prevents the binding of the inducer to the repressor
(D) Glucose transport-mediated inhibition of lac permease

2021

**74.** An E. coli cell contains a nonsense mutation early in the lacZ coding sequence. This also causes dramatically reduced expression of lacY and lacA. This phenomenon is an example of:
(A) A polar mutation
(B) Constitutive expression
(C) Inducer exclusion
(D) Catabolite repression

2012

**75.** A high concentration of glucose in the growth medium directly causes which intracellular response related to the lac operon?
(A) An increase in intracellular cAMP levels
(B) Decreased cAMP, preventing CAP-promoter binding
(C) The proteolytic degradation of the lac repressor
(D) The phosphorylation of the β-galactosidase enzyme

2021

**76.** Identify the INCORRECT statement regarding the dual control of the lac operon.
(A) The lac repressor exerts negative control
(B) Maximum transcription occurs when both glucose and lactose are highly abundant
(C) The CAP-cAMP complex exerts positive control
(D) The operon is shut off when glucose is present and lactose is absent

2004

**77.** Comparing the states of the lac operon, which condition yields the highest rate of transcription?
(A) Low glucose, high lactose
(B) High glucose, low lactose
(C) Low glucose, low lactose
(D) High glucose, high lactose

2013

**78.** Identify the correct pair involving the lac operon components.
(A) lacY : encodes transacetylase
(B) CAP : binds to the operator
(C) lacI : encodes the operon's repressor
(D) Operator : binding site for RNA polymerase

2006

**79.** What structural change occurs in the lac repressor upon binding allolactose?
(A) It polymerizes into a large filament
(B) It is cleaved into two inactive halves
(C) It binds directly to RNA polymerase
(D) Conformational shifts reduce its operator affinity

2024

**80.** Consider the following statements regarding the lac operon:
I. The CAP protein requires cAMP to bind efficiently to its recognition site.
II. A mutation in the operator sequence

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Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I and II only

2017

**141.** By definition, an aminoacyl-tRNA synthetase is an enzyme that:
(A) Covalently attaches the correct amino acid to its corresponding tRNA
(B) Synthesizes tRNA molecules
(C) Synthesizes amino acids
(D) Cleaves the amino acid from the tRNA during translation

2005

**142.** What is the first reaction step catalyzed by aminoacyl-tRNA synthetase during the charging of a tRNA?
(A) Transfer of the amino acid to the 3'-OH of the tRNA
(B) Proofreading of the amino acid
(C) Activation of the amino acid with ATP to form an aminoacyl-AMP intermediate
(D) Attachment of the amino acid to the anticodon loop

2009

**143.** Class I and Class II aminoacyl-tRNA synthetases differ fundamentally in their initial attachment site on the terminal adenosine of the tRNA. Where does Class I initially attach the amino acid?
(A) The 3'-hydroxyl group
(B) The 5'-phosphate group
(C) The anticodon loop
(D) The 2'-hydroxyl group

2003

**144.** An aminoacyl-tRNA synthetase accidentally activates an incorrect, structurally similar amino acid. The enzyme corrects this error utilizing a specific hydrolytic site. This mechanism is known as:
(A) Ribosomal proofreading
(B) Kinetic proofreading
(C) Double-sieve proofreading
(D) Nonsense-mediated decay

2015

**145.** In the "double-sieve" mechanism, how does the editing site distinguish the incorrect amino acid from the correct one?
(A) The editing site accommodates the correct amino acid and hydrolyzes it
(B) It accommodates only incorrect amino acids smaller than the correct one
(C) The editing site relies on base-pairing with the tRNA anticodon
(D) The editing site measures the electrical charge of the amino acid

2005

**146.** Identify the INCORRECT statement regarding aminoacyl-tRNA synthetases.
(A) There is generally one synthetase for each of the 20 standard amino acids
(B) The attachment of an amino acid to tRNA is an energy-consuming process
(C) Synthetases recognize their specific tRNAs using both the anticodon and the acceptor stem
(D) Class II synthetases typically function as monomers and attach the amino acid to the 2'-OH

2007

**147.** Comparing the activation and transfer steps of aminoacylation, which high-energy bond is formed during the transfer step?
(A) An ester bond between the amino acid carboxyl group and the ribose of the terminal adenine
(B) A peptide bond
(C) A phosphoester bond
(D) A phosphodiester bond

NODIA

2022

**148.** Identify the correct pair regarding aminoacyl-tRNA synthetases.
(A) Transfer step : hydrolyzes GTP
(B) Class I synthetase : attaches amino acid to the 3'-OH initially
(C) Activation step : releases pyrophosphate (PPi)
(D) Editing site : covalently attaches the amino acid to tRNA

2006

**149.** Why is the accuracy of aminoacyl-tRNA synthetases absolutely critical for faithful translation?
(A) The ribosome incorporates whatever amino acid is attached
(B) The ribosome has an intrinsic ability to check if the amino acid matches the codon
(C) Synthetases are also responsible for forming peptide bonds
(D) Incorrect charging prevents the mRNA from binding to the ribosome

be rejoined by standard cellular enzymes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2021

**211.** Molecular chaperones like the Hsp70 class assist in protein folding primarily by:
(A) Binding hydrophobic regions to prevent aggregation
(B) Forming a barrel-like structure where proteins fold in isolation
(C) Covalently modifying the protein with stabilizing chemical groups
(D) Cleaving misfolded domains

2011

**212.** The GroEL-GroES complex in E. coli represents which specific class of molecular chaperones?
(A) Hsp90
(B) Small heat shock proteins
(C) Chaperonins
(D) Ubiquitin ligases

2002

**213.** How does the GroEL-GroES complex utilize energy to facilitate protein folding?
(A) ATP hydrolysis for conformational cycles
(B) It uses the proton motive force to fold the protein
(C) It requires GTP to synthesize peptide bonds
(D) It operates completely independent of any energy source

2007

**214.** A mutation prevents a cell from synthesizing the GroES co-chaperonine. What is the immediate direct consequence for the GroEL folding cycle?
(A) GroEL cannot bind to unfolded proteins
(B) GroEL degrades all target proteins immediately
(C) GroEL converts into a protease
(D) The chamber lid cannot seal the environment

2005

**215.** When a protein remains persistently misfolded despite the action of chaperones, the cell typically channels it into which quality control pathway?
(A) The lysosomal exocytosis pathway
(B) The ubiquitin-proteasome system
(C) The Golgi secretion pathway
(D) The nuclear import pathway

2006

**216.** Identify the INCORRECT statement regarding protein folding and quality control.
(A) Hsp70 operates closely with co-chaperones like Hsp40
(B) Chaperonins are barrel-shaped complexes
(C) The 26S proteasome degrades proteins into small peptide fragments through multiple cleavage sites
(D) ATP binding and hydrolysis regulate the affinity of Hsp70 for its substrates

2006

**217.** Comparing Hsp70 and GroEL, which characteristic distinguishes GroEL's mechanism of action?
(A) It binds to hydrophobic patches
(B) It acts co-translationally as the protein emerges from the ribosome
(C) It requires ATP
(D) It provides a physically enclosed cage or chamber for the entire protein to fold

2015

**218.** Identify the correct pair involving protein quality control.
(A) GroES : acts as the "lid" for the GroEL complex
(B) 20S core particle : responsible for ubiquitin recognition
(C) 19S regulatory particle : contains the proteolytic active sites
(D) Hsp70 : forms a 14-subunit ring structure

2023

**219.** What specific structural signal on a misfolded protein typically targets it for ubiquitination by quality control E3 ligases?
(A) An N-terminal signal sequence
(B) Prolonged exposure of buried hydrophobic patches
(C) A poly(A) tail
(D) A highly acidic C-terminus

2024

**220.** Match the items in List I with those in List II.
List I: (Chaperone or quality control component)
P. Hsp70
Q. GroEL
R. 26S Proteasome
S. Ubiquitin
List II: (Functional property)
1. Small protein covalently attached as a degradation signal
2. Monomeric chaperone binding hydrophobic stretches

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2019

**160.** The following question consists of two statements—Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): The ribosome is fundamentally considered a ribozyme.
Reason (R): High-resolution crystal structures reveal that the active site for peptide bond formation is devoid of proteins and consists entirely of RNA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**161.** The Shine-Dalgarno sequence in prokaryotic mRNA is characterized by:
(A) A pyrimidine-rich sequence located downstream of the start codon
(B) A purine-rich sequence located upstream of the start codon
(C) A specific sequence of proteins bound to the mRNA
(D) A GC-rich hairpin loop

2016

**162.** What is the primary role of the initiator tRNA (fMet-tRNAfMet) in prokaryotic translation?
(A) It recognizes the stop codon
(B) It binds directly to the A site to initiate translation
(C) It synthesizes the Shine-Dalgarno sequence
(D) It delivers formylmethionine to the P site at the start codon

2019

**163.** During prokaryotic translation initiation, which base-pairing interaction is responsible for positioning the ribosome correctly on the mRNA?
(A) The 16S rRNA base-pairing with the Shine-Dalgarno sequence
(B) The 23S rRNA base-pairing with the start codon
(C) The tRNA anticodon base-pairing with the Shine-Dalgarno sequence
(D) The 5S rRNA base-pairing with the poly(A) tail

2006

**164.** A mutation in the gene encoding Initiation Factor 3 (IF3) would most likely cause:
(A) Failure to form peptide bonds
(B) Inability of the ribosome to translocate
(C) Premature association of the 30S and 50S subunits before mRNA binding
(D) Rapid release of the mRNA from the ribosome

2025

**165.** The hydrolysis of GTP bound to Initiation Factor 2 (IF2) directly causes:
(A) IF2 release and stable joining of the large 50S subunit
(B) The binding of the mRNA to the 30S subunit
(C) The activation of the amino acid
(D) The termination of translation

2011

**166.** Identify the INCORRECT statement regarding prokaryotic translation initiation.
(A) $IF_1$ binds to the A site to prevent premature tRNA entry
(B) The start codon is typically AUG, but occasionally GUG or UUG
(C) The fMet-tRNAfMet is the only aminoacyl-tRNA capable of directly entering the P site
(D) The initiator tRNA binds to the A site of the small subunit

2012

**167.** Comparing the initiation factors, what is the specialized role of IF2?
(A) It keeps the ribosomal subunits dissociated
(B) It acts as a GTPase that escorts the initiator tRNA to the P site
(C) It blocks the A site
(D) It binds the mRNA

2001

**168.** Identify the correct pair regarding prokaryotic translation initiation.
(A) fMet : attached to all tRNAs carrying methionine
(B) IF3 : hydrolyzes ATP
(C) Shine-Dalgarno sequence : base pairs with the 3' end of 16S rRNA
(D) 70S initiation complex : lacks the 50S subunit

NODIA

2015

**169.** What ensures that the translation machinery in prokaryotes can initiate translation at multiple distinct start sites on a polycistronic mRNA?
(A) The presence of a 5' cap on each cistron
(B) The ability of the ribosome to scan from the 5' end
(C) The use of internal ribosome entry sites (IRES)

(D) Independent Shine-Dalgarno sequences for each cistron

2022

**170.** Match the items in List I with those in List II.
List I: (Prokaryotic initiation factor)
P. $IF_1$
Q. IF2
R. IF3
S. fMet-tRNAfMet
List II: (Specific function)
1. GTP-binding protein that brings initiator tRNA to the P site
2. Binds to the A site to prevent incorrect tRNA entry
3. Prevents premature joining of 30S and 50S subunits
4. Provides the first amino acid of the polypeptide
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-4, S-3
(C) P-3, Q-1, R-2, S-4
(D) P-2, Q-3, R-1, S-4

2011

**171.** The cap-dependent scanning mechanism in eukaryotes involves the small ribosomal subunit binding to which structure?
(A) The Shine-Dalgarno sequence
(B) The 5' m7G cap
(C) The poly(A) tail
(D) The 3' UTR

2010

**172.** What is the defining characteristic of the Kozak consensus sequence in eukaryotic mRNAs?
(A) It dictates where the poly(A) tail is added
(B) Optimized AUG context enhancing translation initiation
(C) It acts as a binding site for the rho terminator
(D) It replaces the 5' cap in specialized mRNAs

2001

**173.** The assembly of the eIF4F complex is essential for cap-dependent initiation. Which protein within this complex directly binds the 5' cap?
(A) eIF4E
(B) eIF4A
(C) eIF4G
(D) eIF3

2025

**174.** An mRNA molecule translates efficiently despite having its 5' cap experimentally removed. This mRNA most likely utilizes which alternative initiation mechanism?
(A) Shine-Dalgarno sequence pairing
(B) Rho-dependent protein initiation
(C) Internal Ribosome Entry Site (IRES)
(D) Poly(A) dependent translation

2015

**175.** The formation of the 43S pre-initiation complex involves the association of the 40S subunit with eIFs and a ternary complex. What comprises this ternary complex?
(A) eIF4E, eIF4G, and eIF4A
(B) $IF_1$, IF2, and IF3
(C) The 60S subunit, mRNA, and ATP
(D) eIF2, GTP, and Met-tRNAiMet

2025

**176.** Identify the INCORRECT statement regarding eukaryotic translation initiation.
(A) The initiator tRNA carries unformylated methionine
(B) eIF4A is an RNA helicase that unwinds secondary structure in the 5' UTR
(C) The 48S initiation complex is formed when the 60S subunit joins the 40S subunit
(D) Scanning requires ATP hydrolysis

2022

**177.** Comparing cap-dependent initiation and IRES-mediated initiation, which factor is frequently bypassed or cleaved during viral IRES utilization?
(A) eIF4G
(B) eIF4E
(C) eIF3
(D) The 40S subunit

2024

**178.** Identify the correct pair involving eukaryotic initiation factors.
(A) eIF2 : hydrolyzes ATP to unwind mRNA
(B) eIF4E : binds the Kozak sequence
(C) eIF5 : serves as the principal helicase
(D) eIF4G : serves as a scaffold interacting with eIF4E and PABP

2019

**179.** Once the scanning 40S subunit recognizes the correct AUG codon, which critical event triggers the release of multiple eIFs and allows the 60S subunit to join?
(A) GTP hydrolysis by eIF2 triggered by eIF5
(B) Phosphorylation of the eIF2 complex
(C) Hydrolysis of ATP by the eIF4A factor
(D) Enzymatic cleavage of the target mRNA

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2006

**180.** Select the correct order of events in eukaryotic translation initiation.
(A) 43S PIC formation → eIF4F assembly on mRNA → Scanning → 48S complex formation → 80S assembly
(B) 48S complex formation → Scanning → eIF4F assembly → 80S assembly → 43S PIC formation
(C) eIF4F assembly → 80S assembly → 43S PIC formation → Scanning → 48S complex formation
(D) Scanning → 43S PIC formation → 48S complex formation → eIF4F assembly → 80S assembly

2025

**181.** By definition, what happens during the "decoding" step of translation elongation?
(A) An aminoacyl-tRNA binds to the A site based on codon-anticodon complementarity
(B) The ribosome moves one codon along the mRNA
(C) The peptide bond is formed
(D) The uncharged tRNA is ejected from the E site

2005

**182.** Which prokaryotic elongation factor is responsible for delivering the aminoacyl-tRNA to the A site of the ribosome?
(A) EF-Ts
(B) EF-G
(C) IF2
(D) EF-Tu

2021

**183.** The formation of the peptide bond transfers the growing polypeptide chain from the tRNA in the P site to the amino group of the amino acid in the A site. This reaction is fundamentally a:
(A) Phosphorylation
(B) Nucleophilic attack
(C) Decarboxylation
(D) Deamination

2019

**184.** A point mutation in EF-Tu prevents it from hydrolyzing GTP. What is the direct consequence during translation?
(A) Translocation occurs prematurely
(B) Peptide bond formation occurs exceptionally fast
(C) The aminoacyl-tRNA cannot be released from EF-Tu into the A site, halting elongation
(D) The ribosome falls apart

2002

**185.** During translocation, the movement of the ribosome along the mRNA is driven by the hydrolysis of GTP mediated by which factor?
(A) EF-Tu
(B) EF-Ts
(C) Peptidyl transferase
(D) EF-G (in prokaryotes) or eEF2 (in eukaryotes)

2009

**186.** Identify the INCORRECT statement regarding translation elongation.
(A) Translocation shifts the deacylated tRNA from the P site to the A site
(B) EF-Ts functions as a guanine nucleotide exchange factor (GEF) for EF-Tu
(C) The growing polypeptide chain briefly resides in the A site immediately after peptide bond formation
(D) Eukaryotic $eEF_1A$ is the functional homolog of prokaryotic EF-Tu

2006

**187.** Comparing prokaryotic and eukaryotic elongation factors, which factor acts as the eukaryotic equivalent of EF-G to facilitate translocation?
(A) $eEF_1A$
(B) eEF2
(C) $eEF_1B$
(D) eIF4G

2015

**188.** Identify the correct pair involving elongation processes.
(A) Peptidyl transferase : requires ATP
(B) EF-G : structurally mimics an aminoacyl-tRNA-EF-Tu complex to force translocation
(C) EF-Tu : binds uncharged tRNA
(D) EF-Ts : hydrolyzes GTP

2019

**189.** What specific conformational change in the ribosome is essential for the process of translocation?
(A) The rotation of the small subunit relative to the large subunit (ratcheting)
(B) The complete dissociation of the large and small subunits
(C) The unfolding of the 23S rRNA
(D) The contraction of the exit tunnel

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2021

**150.** Consider the following statements about aminoacyl-tRNA synthetases:

I. They perform a two-step reaction requiring ATP.
II. The "activation site" excludes amino acids larger than the correct one.
III. The "editing site" hydrolyzes incorrectly activated amino acids that are smaller than the correct one.
IV. Class II synthetases generally bind the minor groove of the tRNA acceptor stem.
V. They utilize GTP instead of ATP for the activation step.

Which of the above statement(s) is/are INCORRECT?

(A) I and II
(B) II and III
(C) IV and V
(D) I and IV

2014

**151.** By definition, what is the Svedberg (S) value a measure of?

(A) Molecular weight strictly in Daltons
(B) Sedimentation rate in an ultracentrifuge, influenced by mass, shape, and density
(C) The number of nucleotides in an RNA molecule
(D) The catalytic speed of an enzyme

2002

**152.** The prokaryotic 70S ribosome is composed of which two primary subunits?

(A) 40S and 60S
(B) 20S and 50S
(C) 30S and 50S
(D) 40S and 50S

2011

**153.** Within the functioning ribosome, what is the primary role of the A (Aminoacyl) site?

(A) It harbors the tRNA attached to the growing polypeptide chain
(B) It provides the exit path for the uncharged tRNA
(C) It catalyzes the formation of the peptide bond
(D) Binding the incoming aminoacyl-tRNA for decoding

2007

**154.** A researcher treats bacterial cells with an antibiotic that specifically blocks the peptidyl transferase center. Which ribosomal component is primarily being targeted?

(A) The 23S rRNA
(B) The 16S rRNA
(C) The 30S subunit proteins
(D) The 5S rRNA

2001

**155.** The catalytic activity responsible for peptide bond formation during translation is uniquely performed by:

(A) A ribosomal protein
(B) An RNA molecule (ribozyme) within the large subunit
(C) An aminoacyl-tRNA synthetase
(D) Elongation factor Tu

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2002

**156.** Identify the INCORRECT statement regarding ribosome anatomy.

(A) The eukaryotic 80S ribosome consists of a 40S small subunit and a 60S large subunit
(B) The small subunit contains the peptidyl transferase center
(C) The P (Peptidyl) site holds the tRNA carrying the growing polypeptide chain
(D) The E (Exit) site is where uncharged tRNAs leave the ribosome

2011

**157.** Comparing prokaryotic and eukaryotic ribosomes, which rRNA molecule is present in the large subunit of eukaryotic ribosomes but absent in prokaryotes?

(A) 5.8S rRNA
(B) 5S rRNA
(C) 16S rRNA
(D) 23S rRNA

2006

**158.** Identify the correct pair involving ribosomal components.

(A) 28S rRNA : component of the 40S subunit
(B) 23S rRNA : component of the 60S subunit
(C) 16S rRNA : component of the 30S subunit
(D) 18S rRNA : component of the 50S subunit

2022

**159.** What structural feature of the ribosome ensures that the mRNA is decoded accurately?

(A) The physical distance between the A and P sites
(B) The peptidyl transferase center in the large subunit
(C) The exit tunnel
(D) The decoding center monitoring codon-anticodon binding

I. $RF_1$ recognizes UAG, while RF2 recognizes UGA.
II. Both $RF_1$ and RF2 recognize UAA.
III. Peptidyl transferase shifts to an esterase activity during termination.
IV. IF3 is required after subunit dissociation to keep the 30S subunit from reassociating with the 50S subunit prematurely.
V. RRF operates only in eukaryotic cells.

Which of the above statement(s) is/are correct?
(A) I, II, III and IV
(B) II, III, IV and V
(C) I, III and V
(D) I, II and IV

2015

**201.** By definition, a post-translational modification (PTM) occurs:
(A) During the synthesis of mRNA
(B) Before a protein has folded
(C) Strictly inside the nucleus
(D) After the synthesis of the polypeptide chain is completed

2022

**202.** Which post-translational modification involves the addition of a sugar moiety to the amide nitrogen of an asparagine residue?
(A) Asparagine N-linked glycosylation
(B) Serine O-linked glycosylation
(C) Target protein phosphorylation
(D) Polypeptide ubiquitination

2013

**203.** The reversible phosphorylation of proteins is a major regulatory mechanism. Which class of enzymes is responsible for removing phosphate groups from proteins?
(A) Kinases
(B) Phosphatases
(C) Ligases
(D) Methyltransferases

2011

**204.** A protein undergoes polyubiquitination with chains linked at Lysine-48. What is the most likely biological consequence for this protein?
(A) It is targeted to the cell membrane
(B) Degradation via the 26S proteasome
(C) It becomes highly active as an enzyme
(D) It is secreted from the cell

2021

**205.** The process of proteolytic processing, such as the conversion of proinsulin to insulin, involves which biochemical action?
(A) Enzymatic cleavage of peptide bonds
(B) The addition of lipid anchors
(C) Addition of multiple ubiquitin molecules
(D) Phosphorylation of serine residues

2006

**206.** Identify the INCORRECT statement regarding post-translational modifications.
(A) Phosphorylation most commonly occurs on serine, threonine, and tyrosine residues in eukaryotes
(B) O-linked glycosylation typically occurs on the hydroxyl group of serine or threonine
(C) Acetylation of histones generally correlates with transcriptional activation
(D) Methylation of proteins is often catalyzed by enzymes using S-adenosylmethionine (SAM) as a methyl donor

2019

**207.** Comparing N-linked and O-linked glycosylation, where does the initial core glycosylation of N-linked glycoproteins primarily occur?
(A) In the Golgi apparatus
(B) In the cytoplasm
(C) In the lysosome
(D) In the endoplasmic reticulum (ER)

2012

**208.** Identify the correct pair involving a PTM and its typical functional impact.
(A) Ubiquitination (K48) : protein stabilization
(B) Phosphorylation : always activates the target enzyme
(C) Lipidation : anchors proteins to biological membranes
(D) Acetylation : degrades the protein

2008

**209.** What is the primary role of the E3 ubiquitin ligase in the ubiquitination cascade?
(A) It activates ubiquitin using ATP
(B) Specific target recognition and transfer
(C) It acts as a carrier protein for ubiquitin
(D) It digests the ubiquitinated protein

2008

**210.** The following question consists of two statements—Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Proteolytic processing is generally an irreversible post-translational modification.
Reason (R): The cleavage of peptide bonds by proteases results in the permanent removal of portions of the polypeptide chain, which cannot

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2017

**190.** Arrange the steps of a single translation elongation cycle in the correct sequence.
I. Translocation of the ribosome.
II. Binding of the incoming aminoacyl-tRNA to the A site.
III. Release of the deacylated tRNA from the E site.
IV. Peptide bond formation.
(A) II, IV, I, III
(B) I, II, III, IV
(C) II, I, IV, III
(D) IV, I, II, III

2024

**191.** By definition, a nonsense codon serves what primary role during translation?
(A) It dictates the start of translation
(B) It codes for unusual amino acids like selenocysteine
(C) It causes the ribosome to skip to the next exon
(D) It signals the termination of the polypeptide chain

2006

**192.** In prokaryotes, which Release Factor specifically recognizes the UAA and UAG stop codons?
(A) RF2
(B) RF3
(C) $RF_1$
(D) RRF

2009

**193.** The binding of a Release Factor to the A site triggers the peptidyl transferase center to alter its specificity. What molecule does it utilize to cleave the polypeptide from the tRNA?
(A) ATP
(B) A water molecule
(C) A specific amino acid
(D) Pyrophosphate

2023

**194.** A failure in the function of the Ribosome Recycling Factor (RRF) in bacteria would most directly cause:
(A) Failure to recognize stop codons
(B) Permanent attachment of the polypeptide to the tRNA
(C) Premature termination of translation
(D) Stalled 70S ribosome accumulation after termination

2019

**195.** The eukaryotic release factor $eRF_1$ possesses a highly distinctive functional property. What is it?
(A) It recognizes all three stop codons (UAA, UAG, UGA) on its own
(B) It relies entirely on a separate RNA molecule for codon recognition
(C) It forms a peptide bond with the nascent chain
(D) It serves as a GTPase

2022

**196.** Identify the INCORRECT statement regarding translation termination.
(A) Release factors are proteins that mimic the shape of a tRNA molecule
(B) RF3 in prokaryotes functions to release $RF_1$ or RF2 from the ribosome
(C) Eukaryotes require three distinct release factors ($eRF_1$, eRF2, eRF3) to recognize the three stop codons
(D) Termination does not involve the addition of an amino acid

2014

**197.** Comparing prokaryotic and eukaryotic termination, what role does eRF3 play that is analogous to bacterial RF3?
(A) It acts as a GTPase that stimulates the termination process and factor release
(B) It recognizes the UGA codon
(C) It cleaves the polypeptide chain
(D) It recycles the ribosome

2014

**198.** Identify the correct pair involving translation termination components.
(A) $eRF_1$ : binds to the E site
(B) RRF : recognizes the UAG codon
(C) $RF_1$ : acts as a GTPase
(D) RF2 : recognizes UAA and UGA

2002

**199.** What crucial role does EF-G play during the ribosome recycling phase in prokaryotes?
(A) It binds the start codon to re-initiate translation
(B) Subunit separation via GTP hydrolysis with RRF
(C) It degrades the mRNA
(D) It ejects the newly synthesized protein

2019

**200.** Consider the following statements about translation termination and recycling:

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3. Large multimeric complex that degrades polyubiquitinated proteins
4. Double-ring chaperonin complex providing a folding chamber

(A) P-2, Q-4, R-3, S-1
(B) P-4, Q-2, R-1, S-3
(C) P-2, Q-3, R-4, S-1
(D) P-1, Q-4, R-2, S-3

2025

**221.** The Signal Hypothesis, proposed by Blobel and Sabatini, explains how proteins are targeted to the:
(A) Endoplasmic reticulum (ER)
(B) Nucleus
(C) Mitochondria
(D) Peroxisome

2015

**222.** What defines the typical structure of an ER-targeting signal sequence?
(A) A sequence of basic amino acids at the C-terminus
(B) A specific pattern of phosphorylated serines
(C) 6-12 hydrophobic residues near the N-terminus
(D) An exposed β-barrel

2014

**223.** During co-translational translocation into the ER, what binds to the signal sequence as soon as it emerges from the ribosome?
(A) The Sec61 translocon
(B) Signal peptidase
(C) BiP
(D) Signal Recognition Particle

2017

**224.** A drug inhibits the GTPase activity of the SRP receptor. What is the expected consequence for ER protein targeting?
(A) The ribosome will not bind to the ER membrane
(B) The SRP will not bind the signal sequence
(C) Stalled transfer from SRP to the translocon
(D) The signal peptide will be cleaved prematurely

2015

**225.** Post-translational import of proteins into mitochondria requires the protein to be in which state?
(A) Fully folded and modified
(B) Unfolded, stabilized by chaperones like Hsp70
(C) Polyubiquitinated
(D) Complexed with a ribosome

2002

**226.** Identify the INCORRECT statement regarding protein targeting.
(A) Mitochondrial targeting signals are often amphipathic α-helices
(B) The Sec61 complex forms the aqueous channel of the ER translocon
(C) SRP binding halts or slows down translation until the ribosome reaches the ER
(D) Nuclear localization signals (NLS) are typically not cleaved off after import

2024

**227.** Comparing nuclear import and ER import, what is a fundamental difference in how proteins cross the respective membranes?
(A) Nuclear import uses a protein channel (NPC) that accommodates fully folded proteins; ER import typically requires unfolded linear chains passing through the translocon
(B) Nuclear import is co-translational; ER import is strictly post-translational
(C) Nuclear import requires signal cleavage; ER import does not
(D) Nuclear import does not require energy

2012

**228.** Identify the correct pair involving protein targeting components.
(A) SRP : targets proteins to the nucleus
(B) TOM complex : imports proteins into the peroxisome
(C) Sec translocon : translocates proteins across the ER membrane
(D) Importin : binds the signal recognition particle

2004

**229.** What is the specific role of the Ran-GTPase cycle in nuclear transport?
(A) Establish a gradient directing transport flow
(B) It provides the energy for unfolding proteins
(C) It acts as a translocon channel
(D) It cleaves the nuclear export signal

2013

**230.** Select the correct order of events for co-translational targeting to the ER.
(A) SRP binds signal sequence → Translation pauses → SRP binds receptor → Ribosome transferred to translocon → Translation resumes
(B) SRP binds receptor → Translation pauses → SRP binds signal sequence → Translation

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resumes → Ribosome transferred to translocon

(C) Translation pauses → SRP binds signal sequence → Ribosome transferred to translocon → SRP binds receptor → Translation resumes

(D) Ribosome transferred to translocon → SRP binds signal sequence → Translation pauses → SRP binds receptor → Translation resumes

2002

**231.** By definition, translational control at the global level often involves modifying components of the initiation machinery. Which factor is most commonly targeted?

(A) $eEF_1A$

(B) eIF2

(C) $eRF_1$

(D) eIF4A

2017

**232.** Under conditions of cellular stress (e.g., viral infection or amino acid starvation), specific kinases phosphorylate the α subunit of eIF2. What is the immediate effect?

(A) eIF2 gains increased ability to hydrolyze ATP

(B) eIF2 rapidly transports all mRNAs to the proteasome

(C) Inhibition of the exchange factor eIF2B

(D) eIF2 induces widespread mRNA capping

2025

**233.** The Iron Response Element (IRE) located in the 5' UTR of ferritin mRNA regulates translation. What binds to this IRE under low iron conditions?

(A) The 40S ribosomal subunit directly

(B) eIF4G, which enhances translation

(C) Free iron ions

(D) IRP protein blocking PIC scanning

2007

**234.** A mutation in the IRE of the transferrin receptor mRNA (located in its 3' UTR) prevents IRP from binding. What will happen to the mRNA under low iron conditions?

(A) Rapid nuclease degradation from lack of binding

(B) It will be highly stabilized and translated efficiently

(C) It will be exported to the mitochondria

(D) It will undergo immediate alternative splicing

2019

**235.** The major pathway for mRNA decay in eukaryotes is initiated by which specific biochemical event?

(A) Removal of the 5' cap

(B) Deadenylation (shortening of the tail)

(C) Cleavage by the exosome

(D) Excision of the start codon

2016

**236.** Identify the INCORRECT statement regarding translational control and mRNA decay.

(A) Global regulation affects almost all mRNAs, while specific regulation targets individual mRNAs

(B) Decapping enzymes remove the 5' cap only after the poly(A) tail is significantly lengthened

(C) The 3' to 5' degradation of mRNA is carried out by a multiprotein complex called the exosome

(D) Phosphorylation of eIF2-α is a key mechanism of the integrated stress response

2010

**237.** Comparing the two main pathways of eukaryotic mRNA degradation, what distinguishes the 5'-to-3' pathway from the 3'-to-5' pathway?

(A) 5'-to-3' involves Dcp1/Dcp2 and Xrn1 activity

(B) The 5'-to-3' pathway requires deadenylation first; the 3'-to-5' pathway does not

(C) The 3'-to-5' pathway is entirely independent of the poly(A) tail length

(D) Both pathways utilize the exosome

NODIA

2006

**238.** Identify the correct pair regarding translational control mechanisms.

(A) eIF2-α phosphorylation : increases global translation rate

(B) Decapping : carried out by the exosome

(C) Ferritin IRE regulation : controls translation initiation based on iron levels

(D) P-bodies : sites of active translation

2012

**239.** What is the primary function of Processing bodies (P-bodies) in eukaryotic cells?

(A) They are the primary sites of ribosome assembly

(B) They are nuclear structures where splicing occurs

(C) They synthesize the poly(A) tail

(D) Cytoplasmic foci for mRNA decay and storage

2011

**240.** Arrange the typical sequence of events in the major deadenylation-dependent mRNA decay pathway.
I. Decapping by Dcp1/Dcp2.
II. 5' to 3' exonucleolytic degradation by Xrn1.
III. Shortening of the poly(A) tail by deadenylases.
IV. Loss of PABP binding.
(A) III, IV, I, II
(B) I, II, III, IV
(C) III, I, IV, II
(D) IV, III, II, I

2019

**241.** Which of the following best describes the role of the TATA box in eukaryotic transcription?
(A) It determines the reading frame for translation
(B) It acts as the primary binding site for TFIID to establish the transcription initiation site
(C) It terminates transcription
(D) It regulates RNA splicing

2004

**242.** In the context of eukaryotic gene regulation, what happens when an insulator element is placed between an enhancer and a promoter?
(A) The enhancer's activity is doubled
(B) The promoter begins bidirectional transcription
(C) The insulator acts as a new promoter
(D) Enhancer-promoter activation is blocked

2004

**243.** The enzymatic activity of histone acetyltransferases (HATs) directly leads to:
(A) Relaxed chromatin and easier transcription
(B) A more compact chromatin structure and repressed transcription
(C) The immediate degradation of histones
(D) The methylation of DNA

2002

**244.** A point mutation changes a UAC codon to a UAA codon in an mRNA. What type of mutation is this?
(A) Missense mutation
(B) Silent mutation
(C) Nonsense mutation
(D) Frameshift mutation

2012

**245.** During translation termination, the hydrolysis of the ester bond linking the polypeptide to the tRNA in the P site is triggered by:
(A) The specific protein release factor
(B) An incoming aminoacyl-tRNA
(C) EF-G
(D) eIF4E

2024

**246.** Identify the INCORRECT statement about post-translational processing.
(A) Signal peptidase removes signal sequences in the ER
(B) Glycosylation can affect protein stability and cell-cell recognition
(C) Molecular chaperones require ATP to fold some proteins
(D) Ubiquitination targets proteins for degradation within the lysosome

2008

**247.** Comparing transcriptional and translational control, which offers the cell the fastest response to an environmental change?
(A) Transcriptional control, because it makes RNA immediately
(B) Translational control using existing mRNA
(C) They operate at exactly the same speed
(D) Neither; only post-translational modification provides a rapid response

2006

**248.** Identify the correct pair matching an RNA with its primary processing event.
(A) tRNA : Addition of a 5' cap
(B) rRNA : Polyadenylation
(C) Pre-mRNA : Addition of a 3' poly(A) tail
(D) snRNA : Translation

2015

**249.** What is the fundamental cause of the "wobble" effect at the third position of a codon?
(A) The third position is not read by the ribosome
(B) The mRNA mutates at the third position during translation
(C) Aminoacyl-tRNA synthetases do not recognize the third position
(D) Non-standard bonding at the 5' anticodon

2014

**250.** Consider the final stages of the central dogma:
I. Protein folding is often co-translational.
II. Misfolded proteins are immediately exported from the cell.
III. The proteasome requires proteins to be tagged with polyubiquitin for recognition.
IV. Phosphorylation is an irreversible post-translational modification.

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responsible for protein dimerization, this highly basic region is essential for making direct contact with the DNA and recognizing the specific nucleotide sequence.

**65.** (B) Disrupted zinc coordination and DNA binding loss
The structural integrity of a $C_2H_2$ zinc finger depends entirely on the coordination of the zinc ion by the specific cysteine and histidine residues. Replacing one of these critical histidines with an alanine would prevent the motif from properly folding into its functional shape. This disruption would lead to the loss of the recognition helix's ability to bind to the DNA.

**66.** (D) Leucine zippers bind DNA directly using the leucine-rich dimerization helix
Leucine zippers are primarily dimerization motifs that bring two protein subunits together. The leucine-rich α-helix itself does not make direct contacts with the DNA bases for sequence recognition. Instead, the actual DNA binding is performed by an adjacent region of the protein that is rich in basic amino acids, which is why these factors are often referred to as bZIP proteins.

**67.** (A) Coordinates zinc via four cysteines and mediates dimerization
The C4 zinc finger is a distinct class of metal-binding motifs commonly found in nuclear receptors. Unlike the $C_2H_2$ variety, it coordinates a zinc ion using four cysteine residues. These motifs often function in pairs within a single protein or through dimerization of two subunits. They play a dual role by facilitating both sequence-specific DNA binding and the necessary protein-protein interactions.

**68.** (C) $C_2H_2$ zinc finger : Found as tandem repeats inserting α helices into the major groove
The $C_2H_2$ zinc finger motif is frequently found as a series of tandem repeats within a transcription factor. Each individual finger utilizes an α-helix to recognize a specific three-base-pair sequence in the major groove of the DNA. By linking multiple fingers together, the protein can recognize longer and more specific DNA sequences, providing a high degree of precision in gene regulation.

NODIA

**69.** (A) The major groove has more unique recognition patterns
Transcription factors typically interact with the major groove of DNA because it offers a more diverse and accessible landscape of chemical information. Each base pair in the major groove presents a unique pattern of hydrogen bond donors, acceptors, and hydrophobic groups. This allows proteins to distinguish between different nucleotide sequences with high specificity, whereas the minor groove provides much less discriminatory information.

**70.** (B) P-3, Q-1, R-2, S-4
Different DNA-binding motifs utilize unique structural features to recognize genetic sequences. The helix-turn-helix motif uses two α-helices without metal ions, while zinc fingers rely on zinc coordination by specific residues like cysteines and histidines. Leucine zippers feature periodic leucines for dimerization, and basic helix-loop-helix motifs use helices separated by a loop. These varied structures allow for precise and diverse regulation.

**71.** (B) It acts as an inducer by altering repressor conformation
Allolactose is an isomer of lactose that is produced in small amounts by β-galactosidase when lactose is present in the cell. It serves as the natural inducer for the lac operon. By binding to the lac repressor, allolactose induces a conformational change that reduces the repressor's affinity for the operator, allowing RNA polymerase to initiate transcription of the operon's genes.

**72.** (C) lacZ
The lacZ gene is the first structural gene of the lac operon and encodes the enzyme β-galactosidase. This enzyme is responsible for cleaving the disaccharide lactose into its component monosaccharides, glucose and galactose. Additionally, it catalyzes the conversion of lactose into allolactose, which acts as the inducer that regulates the expression of the entire operon based on lactose availability.

**73.** (D) Glucose transport-mediated inhibition of lac permease
Inducer exclusion is a mechanism of catabolite repression where the presence of glucose inhibits the transport of other sugars into the cell. In the lac operon, the transport of glucose via the phosphotransferase system leads to the

# ANSWERS

**1.** (B) Initiator element
The initiator element is a core promoter component in eukaryotes that overlaps the transcription start site. It plays a critical role in determining the precise location of the start of transcription, especially in promoters that lack a TATA box. By providing a docking site for general transcription factors, it ensures accurate and efficient RNA polymerase recruitment during initiation.

**2.** (D) TATAAT
The Pribnow box is an essential DNA sequence located within the promoter region of prokaryotic genes. This hexameric sequence serves as a critical recognition site for the sigma factor of RNA polymerase, facilitating the unwinding of the DNA double helix. It is situated approximately ten base pairs upstream of the transcription start site in bacterial genomes.

**3.** (A) It is centered approximately 35 base pairs upstream of the transcription start site
The -35 sequence is a conserved hexameric motif found in bacterial promoters that is recognized by the sigma subunit of the RNA polymerase holoenzyme. It is positioned roughly thirty-five base pairs upstream of the transcription start site. This sequence works in conjunction with the -10 region to ensure stable binding and proper orientation of the polymerase.

**4.** (C) GC box
The GC box is a common proximal promoter element found in eukaryotic genes, typically recognized by the Sp1 transcription factor. Unlike prokaryotic promoters, which rely primarily on the -10 and -35 sequences, eukaryotic promoters utilize a variety of regulatory elements like GC boxes and CAAT boxes to modulate transcription efficiency and facilitate the assembly of the pre-initiation complex.

**5.** (A) Both the -10 and -35 sequences
In prokaryotes, the sigma factor is the subunit of the RNA polymerase holoenzyme responsible for promoter recognition. It binds specifically to the -10 and -35 consensus sequences to position the core enzyme at the correct start site. This interaction is necessary for the formation of a stable closed complex and the subsequent transition to an open complex.

**6.** (D) All eukaryotic promoters require a TATA box for transcription initiation
Eukaryotic promoters exhibit significant diversity in their core architecture. While the TATA box is a well-known element used to recruit the transcription machinery, it is not present in all genes. Many housekeeping genes and developmental genes utilize alternative elements like the initiator or the downstream promoter element to direct RNA polymerase II to the correct transcription start site.

**7.** (B) To modulate the frequency of transcription initiation
Proximal promoter elements, such as the CAAT box and GC box, are located upstream of the core promoter. These sequences bind specific transcription factors that interact with the basal transcription machinery to regulate the frequency and rate of transcription initiation. Unlike the core promoter, which defines the start site, these elements primarily function to modulate the overall expression level.

**8.** (C) -35 sequence : prokaryotic core promoter
The -35 sequence is a fundamental component of the prokaryotic core promoter, acting as a recognition site for the sigma subunit. This hexameric element is located upstream of the Pribnow box and is essential for the initial binding of RNA polymerase. Proper spacing between these promoter elements is critical for efficient recruitment of the enzyme and the start of transcription.

**9.** (D) TATA box
The TATA box is a conserved DNA sequence found in the core promoter of many eukaryotic genes. It is specifically recognized and bound by the TATA-binding protein, which is a subunit of the general transcription factor TFIID. This binding event is the first step in assembling the pre-initiation complex, serving as a platform for other factors and polymerase.

NODIA

**10.** (A) Both A and R are true and R is the correct explanation of A.
The prokaryotic RNA polymerase holoenzyme consists of the core enzyme and a sigma factor. The core enzyme possesses the catalytic activity for RNA synthesis but cannot recognize promoters independently. The sigma factor provides the necessary specificity by binding to the -10 and -35 sequences. Therefore, the holoenzyme is required for accurate and specific initiation of transcription at promoter sites.

**11.** (B) B
The β subunit is a critical component of the prokaryotic RNA polymerase core enzyme, forming part of the active site. It is directly involved in the catalytic process of forming phosphodiester bonds during RNA synthesis. Along with the β-prime subunit, it creates the DNA-binding channel and coordinates the addition of ribonucleotides to the growing RNA chain during elongation.

**12.** (B) Polymerase I synthesizes rRNA, while polymerase II synthesizes mRNA
Eukaryotic cells utilize distinct RNA polymerases for the synthesis of different classes of RNA. RNA polymerase I is specialized for the transcription of ribosomal RNA genes in the nucleolus, producing the large 45S precursor. In contrast, RNA polymerase II is responsible for the synthesis of messenger RNA and several small nuclear RNAs, which are critical for protein-coding gene expression.

**13.** (A) RNA Polymerase IV
RNA polymerase IV is a specialized enzyme found in plants that plays a vital role in the RNA-directed DNA methylation pathway. It synthesizes short non-coding RNAs that serve as precursors for small interfering RNAs. These molecules are essential for maintaining genomic stability and regulating gene expression through the formation of heterochromatin and the silencing of repetitive elements and transposons.

**14.** (C) Eukaryotic RNA polymerase II
A-amanitin is a potent toxin produced by certain mushrooms that differentially inhibits eukaryotic RNA polymerases. RNA polymerase II is highly sensitive to this compound, with very low concentrations being sufficient to block its activity. This sensitivity allows researchers to distinguish its function from RNA polymerase I, which is insensitive, and RNA polymerase III, which shows moderate sensitivity depending on the species.

**15.** (D) It directs transcription of heat shock protein genes
Sigma-32, encoded by the rpoH gene, is an alternative sigma factor in E. coli that is activated during heat stress. It redirects the RNA polymerase holoenzyme to specific promoters of heat shock genes. These genes encode chaperones and proteases that help the cell cope with protein denaturation and damage caused by elevated temperatures, ensuring survival during environmental stress conditions.

**16.** (C) The omega subunit in prokaryotes is essential for the catalytic addition of nucleotides
The omega subunit is a small component of the prokaryotic RNA polymerase core enzyme. Rather than having a direct catalytic role in nucleotide addition, its primary function is to assist in the proper assembly and stability of the enzyme complex. It facilitates the folding of the β-prime subunit and ensures that the core enzyme remains functionally intact for transcription processes.

**17.** (B) RNA Polymerase III
RNA polymerase III is one of the three main RNA polymerases in eukaryotic cells. It is specifically responsible for transcribing a variety of small, stable RNAs, including transfer RNAs and the 5S ribosomal RNA. These molecules are essential components of the translation machinery. The enzyme recognizes internal promoter elements and is moderately sensitive to inhibition by the toxin α-amanitin.

**18.** (D) RNA Polymerase II : snRNA and mRNA
RNA polymerase II is the primary enzyme responsible for the transcription of protein-coding genes into messenger RNA in eukaryotes. In addition to mRNA synthesis, it also transcribes several small nuclear RNAs that are integral components of the spliceosome. The activity of this polymerase is tightly regulated by a wide range of general and specific transcription factors and various promoter elements.

**19.** (A) Enzyme assembly and binding to UP elements
The α subunit of prokaryotic RNA polymerase plays a structural role in the assembly of the

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core enzyme complex. It consists of two domains; the amino-terminal domain is required for dimerization and assembly, while the carboxy-terminal domain can interact with upstream promoter elements or regulatory proteins. This interaction helps to enhance the recruitment of the polymerase and modulate transcriptional activity.

**20.** (A) P-3, Q-1, R-2, S-4
The sensitivity of RNA polymerases to α-amanitin varies across different types. RNA polymerase I is insensitive, while RNA polymerase II is highly sensitive even at very low concentrations. RNA polymerase III exhibits moderate sensitivity, which can vary between species. Bacterial RNA polymerase is entirely insensitive to this toxin, as it is primarily affected by antibiotics like rifampicin that target its subunits.

**21.** (A) GC-rich hairpin loop followed by a run of U residues
Intrinsic termination, also known as rho-independent termination, occurs when RNA polymerase encounters a specific sequence in the DNA. This sequence typically consists of a GC-rich inverted repeat followed by a series of adenine residues. The resulting RNA forms a stable hairpin loop that causes the polymerase to stall, while the weak uracil-adenine hybrid destabilizes the complex, leading to transcript release.

**22.** (D) ATP-dependent RNA-DNA helicase
Rho-dependent termination in prokaryotes requires the rho protein, which acts as a hexameric ATPase. This protein binds to the nascent RNA at a specific site called the rut site and moves along the strand toward the RNA polymerase. Upon reaching the transcription bubble, rho utilizes its ATP-dependent RNA-DNA helicase activity to unwind the hybrid, causing the dissociation of the transcript.

**23.** (B) Pyrophosphate
The elongation of an RNA chain involves the addition of ribonucleoside triphosphates to the 3' hydroxyl end of the growing transcript. This reaction is catalyzed by RNA polymerase and results in the formation of a phosphodiester bond. During this chemical process, a molecule of pyrophosphate is released from the incoming nucleotide, which is subsequently hydrolyzed to provide energy for transcription.

**24.** (C) Rho-dependent termination
The rut site is a sequence on the nascent RNA that serves as the binding platform for the rho protein in prokaryotes. This site is typically rich in cytosine and poor in guanine. If a mutation occurs in this region, the rho protein cannot bind effectively, preventing it from reaching the polymerase and terminating transcription at the appropriate sites.

**25.** (D) RNA-DNA hybrid destabilization and detachment
A sequence of multiple uracil residues at the 3' end of a prokaryotic transcript is a hallmark of intrinsic termination. The base pairs formed between these uracils and the template adenines are exceptionally weak. When combined with the tension created by an upstream hairpin loop, this weak association leads to the spontaneous dissociation of the RNA-DNA hybrid and the polymerase.

**26.** (A) Intrinsic termination requires ATP hydrolysis by a termination factor
Intrinsic termination is a passive process that does not require the involvement of external proteins or the hydrolysis of ATP. It is driven entirely by the formation of a stable GC-rich hairpin in the nascent RNA followed by a poly-U tract. This is in contrast to rho-dependent termination, where the rho factor uses ATP energy to dissociate the transcription complex.

NODIA

**27.** (B) The release of the sigma factor and clearance of the promoter
The transition from transcription initiation to elongation in prokaryotes involves the process of promoter clearance. During this stage, the RNA polymerase synthesizes a short RNA chain and then undergoes a conformational change that involves the release of the sigma factor. This release allows the core enzyme to move away from the promoter and proceed with the processive synthesis of RNA.

**28.** (B) Termination : dissociation of RNA polymerase from the DNA template
Transcription termination is the final phase of the transcription process, where the RNA polymerase dissociates from the DNA template and releases the completed RNA transcript. This event occurs when the enzyme encounters specific termination signals, which may be intrinsic or dependent on specialized proteins.

**47.** (D) They dictate the precise start point and direction of transcription
A promoter is a specific DNA sequence that is essential for defining the exact site where transcription begins and the direction in which the RNA polymerase will move. While enhancers can operate from various distances and orientations, promoters must be positioned immediately upstream of the coding region to correctly position the transcription machinery for the accurate initiation of the RNA transcript.

**48.** (C) Insulator : restricts the action of enhancers
Insulators are crucial regulatory elements that define the limits of enhancer activity. By blocking the interaction between an enhancer and a promoter when positioned between them, they ensure that genes are only activated by their appropriate regulatory signals. This spatial restriction prevents the cross-activation of neighboring genes, which is vital for the maintenance of precise and tissue-specific gene expression patterns.

**49.** (B) Formation of a loop connecting the enhancer-bound activator to the mediator complex at the promoter
Long-distance gene regulation is primarily achieved through the formation of DNA loops. Transcription factors bound to a distal enhancer interact with the mediator complex and general transcription factors at the promoter. This interaction brings these distant DNA regions into physical proximity, creating a bridge that facilitates the recruitment and activation of RNA polymerase II, even across thousands of base pairs.

**50.** (A) II and V
Regulatory elements in eukaryotes have distinct functions. Silencers bind repressor proteins to decrease transcription, not activators to increase it. Furthermore, repressors typically function by modifying chromatin structure or interfering with the basal machinery, rather than by directly degrading RNA polymerase molecules. Therefore, statements describing silencers as activator binding sites or repressors as polymerase-degrading enzymes are factually incorrect.

**51.** (A) It bridges activators and basal machinery
A co-activator is a protein that facilitates transcriptional activation without binding directly to DNA. Instead, it serves as a bridge, interacting with sequence-specific activators bound to enhancers and the general transcription machinery at the promoter. Co-activators often possess enzymatic activities, such as histone acetylation, that modify chromatin structure to make the DNA more accessible for the initiation of transcription.

**52.** (C) A DNA-binding domain and an activation domain
A typical transcriptional activator is a modular protein composed of at least two distinct functional domains. The DNA-binding domain recognizes specific sequences in the promoter or enhancer regions. The activation domain interacts with co-activators, mediator complexes, or the general transcription machinery to promote the assembly of the pre-initiation complex. These domains are often independent and can function when swapped experimentally.

**53.** (A) Histone acetyltransferase (HAT) activity
CBP and p300 are highly related transcriptional co-activators that play a central role in gene regulation. They possess intrinsic histone acetyltransferase activity, which allows them to add acetyl groups to the lysine residues of histone tails. This modification neutralizes the positive charge of the histones, leading to a relaxed chromatin structure that facilitates the binding of transcription factors and polymerase.

**54.** (D) Global transcriptional activation
Histone deacetylases are enzymes that remove acetyl groups from histones, generally leading to chromatin condensation and transcriptional repression. When cells are treated with an inhibitor of these enzymes, the levels of histone acetylation increase globally. This results in a more open and accessible chromatin state across the genome, which typically leads to widespread and global transcriptional activation of many genes.

**55.** (B) Acetyl group removal and chromatin compaction
The recruitment of histone deacetylases by co-repressor complexes leads to the removal of acetyl groups from the lysine residues of histones. This biochemical change increases the positive charge of the histones, strengthening their interaction with the negatively charged DNA. As a result, the chromatin becomes more

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**38.** (D) TFIIE : recruits and stimulates TFIIH
TFIIE is a general transcription factor that joins the pre-initiation complex after RNA polymerase II and TFIIF. It plays a specialized role in recruiting and stimulating the enzymatic activities of TFIIH. Specifically, TFIIE enhances the ATPase, helicase, and kinase functions of TFIIH, which are necessary for DNA unwinding and the phosphorylation of the polymerase carboxy-terminal domain during initiation.

**39.** (B) TFIIF
TFIIF is a general transcription factor that forms a stable complex with RNA polymerase II before it arrives at the promoter. It assists in the recruitment of the polymerase to the TBP-TFIIB-DNA complex. By reducing non-specific binding of the polymerase to DNA and stabilizing the overall assembly, TFIIF ensures the efficient and accurate formation of the pre-initiation complex at promoters.

**40.** (D) IV only
In the process of eukaryotic transcription initiation, TFIIH is the factor responsible for phosphorylating the carboxy-terminal domain of RNA polymerase II. TFIIA is a different general transcription factor that functions primarily to stabilize the binding of TFIID and TBP to the promoter. Therefore, the assertion that TFIIA acts as the primary kinase for this phosphorylation event is biologically incorrect.

**41.** (D) A cis-acting sequence regulating distal transcription
An enhancer is a cis-acting DNA sequence that significantly increases the transcription level of a target gene. These elements can function regardless of their distance or orientation relative to the promoter they regulate. They provide binding sites for specific transcription factors, which then interact with the basal transcription machinery through DNA looping, facilitating the recruitment and activation of RNA polymerase.

**42.** (A) The DNA looping model
The DNA looping model explains how distal enhancers can regulate promoters located far away on the same chromosome. According to this model, the DNA between the enhancer and the promoter bends, allowing the proteins bound at both sites to come into direct physical contact. This looping is often facilitated by architectural proteins and the mediator complex, enabling long-distance transcriptional activation.

**43.** (B) Block enhancers from acting on distal promoters
A genetic insulator is a specialized regulatory element that establishes boundaries within the genome. Its primary function is to prevent an enhancer from inappropriately activating a promoter of an adjacent gene. When positioned between an enhancer and a promoter, the insulator blocks their interaction. Additionally, some insulators act as barriers to prevent the spread of heterochromatin into active gene regions.

**44.** (B) Transcription remains elevated due to orientation-independence
Enhancers are characterized by their ability to function independently of their orientation. If an enhancer sequence is flipped 180 degrees, it will still be recognized by the same transcription factors and can still interact with the target promoter through DNA looping. Consequently, the level of transcription at the associated promoter remains elevated, reflecting the versatile nature of these regulatory elements.

**45.** (A) Histone deacetylases (HDACs)
Silencers are regulatory DNA elements that decrease the transcription of target genes. When repressor proteins bind to these sites, they often recruit co-repressor complexes that contain histone deacetylases. These enzymes remove acetyl groups from histone tails, leading to a more condensed and closed chromatin structure. This modification restricts the access of the transcription machinery to the DNA, resulting in gene silencing.

**46.** (C) Their activity is solely dependent on being located precisely at the -35 position
Insulators do not have a fixed position relative to the transcription start site like the -35 sequence in prokaryotes. Instead, their activity depends on their location relative to other regulatory elements, such as being placed between an enhancer and a promoter. Their role is to establish functional domains in the genome rather than serving as direct components of the core promoter.

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Which of the above statement(s) is/are correct?
(A) I and III
(B) I and II
(C) III and IV
(D) II and IV

**********

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compacted and inaccessible, which effectively represses the initiation of transcription by the cellular machinery.

**56.** (C) Co-activators must possess a specific DNA-binding domain to function
Co-activators are characterized by their lack of a sequence-specific DNA-binding domain. Instead, they function by interacting with other proteins that are already bound to the DNA. Their role is to provide a functional link between site-specific activators and the basal transcription apparatus, or to modify the local chromatin environment through enzymatic activities such as histone acetylation or chromatin remodeling.

**57.** (D) Activators bind DNA; co-activators bridge proteins
Activators and co-activators are both essential for high levels of transcription, but they differ in their interaction with DNA. Activators possess specific domains that allow them to bind directly to promoter or enhancer sequences. In contrast, co-activators do not bind DNA directly; they function by bridging the interaction between the bound activators and the general transcription factors or RNA polymerase.

**58.** (A) Activation domain : Interacts with the basal transcription machinery
The activation domain of a transcription factor is a modular region that facilitates the recruitment of the transcription machinery. It functions by interacting with various proteins, including the mediator complex, general transcription factors, or co-activators. These interactions are necessary to stabilize the pre-initiation complex and promote the start of transcription, thereby translating the binding of an activator into gene expression.

**59.** (B) By interacting with a common co-repressor complex
Many transcriptional repressors achieve gene silencing by recruiting common co-repressor complexes. These complexes often contain multiple subunits with enzymatic activities, such as histone deacetylases, that can modify the chromatin structure of various target genes. By interacting with a shared co-repressor, a single type of repressor protein can efficiently regulate the expression of many different genes across the entire genome.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Histone acetylation by enzymes like CBP/p300 is a key mechanism for activating transcription. The addition of acetyl groups to lysine residues in the histone tails neutralizes their positive charge. This reduces the electrostatic attraction between the histones and the negatively charged phosphate backbone of DNA, leading to a more relaxed chromatin structure. This increased accessibility allows the transcription machinery to initiate.

**61.** (A) An amphipathic α-helix with periodic leucines
The basic Leucine Zipper motif is a common structural feature in many transcription factors. It consists of an amphipathic α-helix where leucine residues appear at every seventh position, creating a hydrophobic face. This arrangement allows two such helices to interact and dimerize through a "zipper-like" hydrophobic interface. Adjacent to this dimerization region is a basic domain that mediates DNA binding.

**62.** (C) $C_2H_2$
The $C_2H_2$ zinc finger is one of the most widespread DNA-binding motifs in eukaryotic transcription factors. It is characterized by the coordination of a single zinc ion by two cysteine and two histidine residues. This coordination stabilizes a small protein fold consisting of an α-helix and a β-sheet. The α-helix typically inserts into the major groove of DNA to recognize specific sequences.

**63.** (D) It recognizes specific base pairs in the major groove
The helix-turn-helix motif is a classic DNA-binding structure found in both prokaryotic and eukaryotic proteins. It consists of two α-helices separated by a short turn. One of these helices, known as the recognition helix, fits into the major groove of the DNA. There, it makes specific hydrogen bonds and van der Waals contacts with the bases, allowing for sequence-specific recognition.

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**64.** (C) DNA binding
The basic Helix-Loop-Helix motif consists of a dimerization domain formed by two helices connected by a flexible loop. Immediately adjacent to the first helix is a region rich in basic amino acids. While the HLH portion is

Successful termination ensures that genes are expressed accurately and that the polymerase is recycled for future use.

**29.** (A) G-C
The termination hairpin in intrinsic termination is stabilized by a GC-rich region within the inverted repeat. Guanine and cytosine form three hydrogen bonds, making their pairing significantly stronger than that of adenine and uracil. This structural stability is essential for the hairpin to physically disrupt the transcription bubble and stall the RNA polymerase, facilitating the dissociation of the nascent RNA.

**30.** (A) I and II
In prokaryotes, transcription termination occurs through two primary mechanisms. Rho-dependent termination requires the rho factor to bind at a rut site on the transcript and move towards the polymerase. Intrinsic termination involves the formation of a GC-rich hairpin followed by a poly-U sequence. Both mechanisms lead to the release of the RNA transcript and the dissociation of the transcription complex.

**31.** (D) TFIIH
TFIIH is a complex general transcription factor that plays multiple roles in eukaryotic transcription initiation. It contains a kinase subunit responsible for phosphorylating the serine residues on the carboxy-terminal domain of RNA polymerase II. This phosphorylation event is a critical trigger for promoter clearance, allowing the polymerase to escape the promoter and transition into the elongation phase of transcription.

**32.** (C) It binds the TATA box and induces a DNA bend
The TATA-binding protein is a central component of the general transcription factor TFIID. It specifically recognizes and binds to the TATA box in the core promoter. Upon binding, the protein induces a dramatic bend in the DNA, which facilitates the recruitment of other general transcription factors and RNA polymerase II, thereby promoting the assembly of the pre-initiation complex.

**33.** (B) General factors and specific activators
The mediator complex is a large, multi-subunit co-activator that serves as a bridge between sequence-specific transcription factors and the basal transcription machinery. It integrates signals from multiple activators and repressors, relaying them to RNA polymerase II and the general transcription factors. This interaction is essential for regulating the efficiency of transcription initiation in response to various cellular and environmental cues.

**34.** (D) TFIIB
Following the binding of TFIID to the TATA box, TFIIB is recruited to the promoter. This factor interacts with both the TATA-binding protein and the DNA, stabilizing the initial complex. It also plays a key role in recruiting the RNA polymerase II-TFIIF complex to the site and helps determine the direction and start site of transcription during initiation.

**35.** (A) Promoter clearance and elongation
The phosphorylation of Serine 5 in the carboxy-terminal domain of RNA polymerase II is catalyzed by the kinase subunit of TFIIH. This modification occurs during the final stages of pre-initiation complex assembly. It signals the transition from initiation to elongation, enabling the polymerase to break its contacts with the promoter-bound factors and begin the processive synthesis of the RNA transcript.

**36.** (C) The mediator complex binds directly to the TATA box to initiate transcription
The mediator complex does not bind directly to the TATA box to initiate transcription. Instead, it functions as a modular scaffold that facilitates communication between distal regulatory elements and the core transcription machinery. It interacts with transcriptional activators bound to enhancers and with the RNA polymerase II holoenzyme, thereby modulating the assembly and activity of the pre-initiation complex at promoters.

**37.** (A) The general transcription factors (e.g., TFIID)
General transcription factors in eukaryotes, such as TFIID, perform a role functionally analogous to the sigma factor in bacteria. While the bacterial sigma factor is a single subunit that directs RNA polymerase to the promoter, eukaryotes require a complex assembly of multiple general factors to recognize promoter elements, recruit the polymerase, and initiate the melting of the DNA double helix.

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inhibition of lac permease. This prevents lactose from entering the cell and being converted to allolactose, thereby keeping the operon repressed even if lactose is present.

**74.** (A) A polar mutation
A polar mutation is a type of mutation where a change in an upstream gene affects the expression of downstream genes in the same operon. In the lac operon, a nonsense mutation in the lacZ gene can cause the premature termination of translation. This often leads to the dissociation of ribosomes and the subsequent degradation of the polycistronic mRNA, reducing the expression of lacY.

**75.** (B) Decreased cAMP, preventing CAP-promoter binding
In the presence of high glucose levels, the activity of adenylate cyclase is reduced, leading to a decrease in the intracellular concentration of cyclic AMP. Since the catabolite activator protein must bind to cAMP to form a complex that activates transcription, low levels of cAMP prevent this positive control. Consequently, the lac operon remains transcribed at only a very basal level.

**76.** (B) Maximum transcription occurs when both glucose and lactose are highly abundant
Maximum transcription of the lac operon does not occur when both glucose and lactose are abundant. Instead, the presence of glucose leads to catabolite repression, keeping cAMP levels low and preventing the activation of the operon by the CAP-cAMP complex. High-level expression is only achieved when glucose is absent, ensuring high cAMP, and lactose is present to remove the lac repressor.

**77.** (A) Low glucose, high lactose
The highest rate of lac operon transcription is achieved under conditions where glucose is low and lactose is high. Low glucose levels lead to high concentrations of cAMP, which binds to the catabolite activator protein and promotes recruitment of RNA polymerase. Simultaneously, high lactose levels produce allolactose, which inactivates the lac repressor and clears the operator for the polymerase to proceed.

**78.** (C) lacI : encodes the operon's repressor
The lacI gene is located upstream of the lac operon and encodes the lac repressor protein. This repressor is constitutively expressed and binds to the operator sequence to prevent transcription in the absence of lactose. When the inducer allolactose is present, it binds to the repressor and causes it to dissociate from the operator, allowing the operon's genes to be transcribed.

**79.** (D) Conformational shifts reduce its operator affinity
When allolactose binds to the lac repressor, it acts as an allosteric effector. This binding event triggers a significant conformational shift in the repressor protein. This change specifically alters the orientation of the DNA-binding domains, drastically reducing the protein's affinity for the operator sequence. As the repressor dissociates from the DNA, the roadblock for RNA polymerase is removed, allowing transcription.

**80.** (A) I and II
The lac operon is controlled by both positive and negative regulatory mechanisms. The CAP protein requires cAMP to bind to the promoter and enhance transcription. Mutations in the operator that prevent repressor binding lead to constitutive expression of the operon. However, lacA actually encodes a transacetylase, not a permease; the permease is encoded by the lacY gene. These factors collectively regulate lactose metabolism.

**81.** (B) Normally on, but turned off by the presence of a co-repressor
A repressible operon is a type of genetic system that is typically active and undergoing transcription under normal cellular conditions. It is regulated by a mechanism where the presence of a specific molecule, called a co-repressor, activates a repressor protein. This activated repressor then binds to the operator to turn off transcription, usually as part of a feedback loop for biosynthetic pathways.

**82.** (D) Tryptophan
In the regulation of the trp operon, tryptophan itself serves as the co-repressor. When the intracellular concentration of tryptophan is high, it binds to the inactive apo-repressor protein. This binding event causes a conformational change that enables the repressor to bind to the operator sequence. This effectively blocks RNA polymerase from initiating transcription, thereby stopping the production of more tryptophan.

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process significantly expands the coding capacity of eukaryotic genomes without increasing the total number of genes, facilitating complex developmental and physiological processes.

**120.** (A) P-3, Q-1, R-2, S-4
Splicing relies on specific consensus sequences and RNA components. The 5' splice site typically begins with a GU dinucleotide, while the 3' splice site usually ends with an AG. The branch point contains an invariant adenine necessary for lariat formation. Catalysis is primarily driven by the U6 snRNA. These elements work in concert within the spliceosome to ensure the precise removal of introns from pre-mRNA.

**121.** (D) RNA catalyzing its excision without a spliceosome
A self-splicing intron is a type of RNA molecule that possesses intrinsic catalytic activity, allowing it to remove itself from a primary transcript without the assistance of the spliceosome or other proteins. These introns are classified as ribozymes because the RNA sequence itself folds into a complex three-dimensional structure that facilitates the transesterification reactions needed for splicing. They are found in diverse organisms and organelles.

**122.** (A) A free guanosine nucleotide or nucleoside
Group I self-splicing introns utilize a free guanosine nucleotide or nucleoside, such as GTP, GDP, GMP, or guanosine, as an external cofactor. The 3' hydroxyl group of this guanosine acts as a nucleophile that attacks the 5' splice site in the first step of the splicing reaction. This guanosine becomes covalently attached to the 5' end of the excised intron after the process is complete.

**123.** (B) An internal branch point adenosine uses its 2'-OH to attack the 5' splice site, forming a lariat
Group II self-splicing introns utilize a catalytic mechanism that is remarkably similar to that of the nuclear spliceosome. The first step is initiated by the 2' hydroxyl group of an internal branch point adenosine, which attacks the 5' splice site. This reaction forms a lariat intermediate. The second step involves the ligation of exons and the release of the intron, demonstrating that RNA can catalyze complex splicing.

**124.** (B) Deamination
The editing of RNA through the conversion of cytidine to uridine is a site-specific process mediated by the enzyme cytidine deaminase. This biochemical reaction involves the removal of an amino group from the cytosine base, transforming it into uracil. This change can alter the coding sequence of the mRNA, such as creating a premature stop codon, which allows for the production of different protein versions.

**125.** (A) Guanine
Adenosine-to-inosine RNA editing is a common modification catalyzed by ADAR enzymes. During translation, the ribosome interprets inosine as if it were guanine. This is because inosine forms base pairs with cytosine, similar to the behavior of guanine. Consequently, an A-to-I change in a coding region can lead to a codon change that results in the incorporation of a different amino acid into the protein.

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**126.** (C) Group I introns generate a lariat intermediate
Group I introns do not form a lariat intermediate during the splicing process. Instead, they use an external guanosine cofactor to initiate the first transesterification reaction, resulting in a linear excised intron. This is a fundamental difference from Group II introns and nuclear pre-mRNA splicing, both of which utilize an internal branch point adenosine to form a characteristic lariat structure as a reaction intermediate.

**127.** (D) Group I introns are excised as linear molecules, Group II as lariats
A major distinction between the two main classes of self-splicing introns lies in the shape of the excised intron. Group I introns are released as linear RNA molecules after splicing is complete. In contrast, Group II introns are excised in the form of a lariat structure, featuring a 2'-5' phosphodiester bond. This difference reflects the distinct nucleophiles used to initiate the first step of each reaction.

**128.** (C) APOB gene : C-to-U editing creates a premature stop codon in the intestine
In humans, the APOB gene undergoes tissue-specific C-to-U RNA editing in the intestine. This modification is catalyzed by the enzyme APOBEC-1, which deaminates a specific cytosine to uracil. This change converts a glutamine codon into a stop codon, resulting in a truncated version of the apolipoprotein B

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**92.** (B) 5'-to-5' triphosphate bridge
The connection between the 7-methylguanosine cap and the first nucleotide of a eukaryotic mRNA is a highly unusual 5'-to-5' triphosphate bridge. Unlike the standard 3'-to-5' phosphodiester bonds that link nucleotides in an RNA chain, this linkage involves the 5' carbons of both ribose sugars. This specific structure is resistant to most cellular exonucleases, which typically recognize and cleave standard 5' ends.

**93.** (A) RNA triphosphatase
The process of mRNA capping begins with the action of the enzyme RNA triphosphatase. This enzyme targets the 5' end of the nascent pre-mRNA, which initially bears a triphosphate group. By removing the terminal gamma phosphate, it leaves a diphosphate group at the 5' end. This modification is necessary for the subsequent attachment of the guanine cap by the enzyme guanylyltransferase.

**94.** (C) Complete absence of the 5' cap structure
Guanylyltransferase is an essential enzyme in the capping pathway that catalyzes the addition of a GMP molecule to the 5' end of the nascent transcript. If this enzyme fails to function, the 7-methylguanosine cap cannot be formed. This lack of a cap makes the mRNA highly vulnerable to rapid degradation by 5' exonucleases and severely impairs its ability to be translated.

**95.** (D) The cap-binding protein eIF4E
In eukaryotes, the 5' cap is directly recognized by the initiation factor eIF4E, which is a component of the eIF4F cap-binding complex. This binding event is a prerequisite for the recruitment of the 40S ribosomal subunit to the mRNA. By facilitating this interaction, the cap structure ensures that translation initiation occurs efficiently and at the correct end of the messenger RNA.

**96.** (C) Cap structures are added post-translationally in the cytoplasm
Capping is a co-transcriptional process that occurs in the nucleus, not a post-translational event in the cytoplasm. The enzymes responsible for cap addition are recruited to the phosphorylated carboxy-terminal domain of RNA polymerase II shortly after the start of transcription. This ensures that every pre-mRNA molecule is protected and correctly processed as it emerges from the transcription machinery during synthesis.

**97.** (B) The cap enables nuclear export and ribosome scanning
Eukaryotic cells utilize a 5' cap for several critical functions that are not required in bacteria. The cap is essential for the efficient export of major mRNA from the nucleus to the cytoplasm through the nuclear pore complex. Furthermore, it facilitates the scanning mechanism of the eukaryotic ribosome, allowing it to locate the correct start codon for the initiation of translation.

**98.** (D) Guanylyltransferase : transfers GMP to the 5' end
Guanylyltransferase is a key enzyme in the mRNA capping process. It catalyzes the transfer of a guanosine monophosphate (GMP) group from GTP to the 5' diphosphate end of the nascent pre-mRNA. This reaction creates the characteristic 5'-to-5' triphosphate linkage. Following this step, a methyltransferase enzyme adds a methyl group to the guanine, completing the formation of the functional cap structure.

**99.** (A) The phosphorylated C-terminal domain
The carboxy-terminal domain of RNA polymerase II serves as a critical coordination hub for pre-mRNA processing. Shortly after transcription begins, the CTD is phosphorylated at specific serine residues. This modification allows it to act as a scaffold that recruits capping enzymes, such as RNA triphosphatase and guanylyltransferase, to the 5' end of the nascent RNA as it first emerges from the polymerase.

**100.** (A) II and V
The mRNA capping process is highly regulated and occurs very early during transcription, typically when the nascent RNA is only twenty to thirty nucleotides long. It is not a post-transcriptional event that follows full synthesis. Additionally, mitochondrial mRNAs in plants do not utilize the 5' cap structure. Therefore, assertions that capping occurs after synthesis or is used in plant mitochondria are factually incorrect.

**101.** (A) AAUAAA
Cleavage and polyadenylation of eukaryotic pre-mRNA are directed by a highly conserved consensus sequence, typically AAUAAA. This

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**155.** (B) An RNA molecule (ribozyme) within the large subunit
The formation of peptide bonds during translation is a catalytic process performed by the large ribosomal subunit. This activity, known as peptidyl transferase, is mediated by the ribosomal RNA rather than by a protein enzyme. This makes the ribosome a ribozyme. Specifically, in bacteria, the 23S rRNA is the key component that positions the substrates and facilitates the nucleophilic attack required for synthesis.

**156.** (B) The small subunit contains the peptidyl transferase center
In the architecture of the ribosome, the small subunit is primarily responsible for the decoding of mRNA, while the large subunit houses the peptidyl transferase center. Therefore, the assertion that the small subunit contains the site for peptide bond formation is incorrect. The large subunit provides the catalytic environment where the growing polypeptide chain is extended by the addition of new amino acids during translation.

**157.** (A) 5.8S rRNA
Eukaryotic and prokaryotic ribosomes share a similar overall structure but differ in their specific rRNA and protein compositions. One notable difference is the presence of the 5.8S rRNA in the large (60S) subunit of eukaryotic ribosomes. This small RNA molecule is hydrogen-bonded to the 28S rRNA and is absent in prokaryotes, where its equivalent sequence is part of the larger 23S rRNA molecule.

**158.** (C) 16S rRNA : component of the 30S subunit
The small 30S subunit of the prokaryotic ribosome is characterized by the presence of the 16S rRNA molecule. This RNA is essential for the structure of the subunit and plays a vital role in the initiation of translation by base-pairing with the Shine-Dalgarno sequence in the mRNA. It also contributes to the decoding center, ensuring that the correct tRNA is selected.

**159.** (D) The decoding center monitoring codon-anticodon binding
The accuracy of translation is maintained by the decoding center, which is located within the small ribosomal subunit. This center monitors the complementarity between the mRNA codon and the tRNA anticodon in the A site. If a correct match is detected, the ribosome undergoes conformational changes that trigger the next steps of elongation, thereby ensuring that the genetic code is faithfully translated into proteins.

**160.** (A) Both A and R are true and R is the correct explanation of A.
The ribosome is fundamentally considered a ribozyme because its central catalytic activity, peptide bond formation, is performed by RNA. High-resolution structural studies have shown that the peptidyl transferase center is composed entirely of ribosomal RNA, with no proteins located close enough to participate directly in the catalysis. This discovery revolutionized our understanding of RNA's role in the most ancient and essential cellular processes.

**161.** (B) A purine-rich sequence located upstream of the start codon
The Shine-Dalgarno sequence is a conserved, purine-rich motif found in the 5' untranslated region of prokaryotic mRNAs. It is typically located about eight nucleotides upstream of the start codon. This sequence is essential for the initiation of translation because it provides a binding site for the small ribosomal subunit, helping to position the ribosome correctly at the start of the coding region.

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**162.** (D) It delivers formylmethionine to the P site at the start codon
In prokaryotes, the initiation of translation requires a specialized initiator tRNA that carries a formylated methionine residue. This fMet-tRNA is specifically recognized by initiation factors and is the only tRNA capable of entering the ribosomal P site directly without first passing through the A site. This ensures that the first amino acid of the new polypeptide is correctly positioned at the start codon.

**163.** (A) The 16S rRNA base-pairing with the Shine-Dalgarno sequence
Translation initiation in prokaryotes is facilitated by a specific base-pairing interaction between the mRNA and the ribosome. The 16S rRNA, which is part of the small 30S subunit, contains a sequence at its 3' end that is complementary to the Shine-Dalgarno sequence on the mRNA. This interaction ensures that the ribosome is accurately aligned with the start codon, allowing translation to begin correctly.

and polyadenylated. Therefore, the assertion that the poly(A) tail is necessary for splicing is false.

**111.** (D) Small nuclear ribonucleoproteins (snRNPs)
The major spliceosome is a large, dynamic ribonucleoprotein complex responsible for removing introns from nuclear pre-mRNAs. Its core components are five small nuclear ribonucleoproteins, known as U1, U2, U4, U5, and U6. Each snRNP consists of a specific small nuclear RNA and a set of associated proteins. These complexes work together to recognize splice sites and catalyze the chemical reactions required for splicing.

**112.** (C) Transesterification
Nuclear pre-mRNA splicing is accomplished through two successive transesterification reactions. In these reactions, a phosphodiester bond is broken, and a new one is formed simultaneously, preserving the overall energy of the bonds. This mechanism allows the intron to be excised and the exons to be ligated together with high precision. These reactions are catalyzed by the RNA components within the active spliceosome complex.

**113.** (B) Lariat structure with 2'-5' phosphodiester bond
The first transesterification reaction in splicing results in the formation of a lariat structure. This occurs when the 2' hydroxyl group of an invariant adenine residue at the branch point attacks the 5' splice site. The resulting bond is a unique 2'-5' phosphodiester linkage. The intron remains attached to the 3' exon in this looped form until the second step of splicing is completed.

**114.** (D) Failure of the first step, preventing lariat formation
The invariant adenine at the branch point sequence is essential for the first step of the splicing process. Its 2' hydroxyl group acts as the nucleophile that attacks the 5' splice site to initiate lariat formation. If this adenine is mutated, the first transesterification reaction cannot occur. This failure prevents the excision of the intron and the subsequent ligation of the neighboring exons.

**115.** (A) U2
Spliceosome assembly is a highly ordered process that begins with the recognition of the 5' splice site by the U1 snRNP. This initial binding event facilitates the subsequent recruitment of the U2 snRNP to the branch point sequence. The interaction between U2 and the branch point is crucial for defining the intron boundaries and preparing the pre-mRNA for the first catalytic step of splicing.

**116.** (C) The first transesterification involves the 3' OH of the upstream exon attacking the 5' splice site
The first transesterification step of splicing involves a nucleophilic attack by the 2' hydroxyl group of the branch point adenosine on the phosphate at the 5' splice site. It is not initiated by the 3' hydroxyl of the upstream exon. That attack occurs during the second transesterification step, where the free 5' exon is ligated to the 3' exon, resulting in the excision of the intron.

**117.** (A) The 3' OH of the free 5' exon
During the second transesterification reaction of splicing, the 3' hydroxyl group of the upstream exon, which was released in the first step, acts as a nucleophile. It attacks the phosphodiester bond at the 3' splice site. This reaction results in the covalent ligation of the two exons and the release of the intron in the form of a lariat structure, completing the process.

**118.** (D) U2 snRNP : binds to the branch point sequence
The U2 snRNP is an essential component of the spliceosome that specifically recognizes and binds to the branch point sequence within an intron. This binding is mediated by base-pairing between the U2 snRNA and the RNA transcript. By accurately positioning the branch point adenosine, U2 prepares the complex for the first catalytic step of splicing, ensuring that the lariat is formed correctly.

**119.** (B) Single genes encoding multiple protein isoforms
Alternative splicing is a versatile regulatory mechanism that allows a single gene to produce multiple distinct protein isoforms. By selectively including or excluding different exons from the mature mRNA, a cell can generate proteins with varied functions, localizations, or stabilities. This

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termination signal in the nucleus, is translated as the amino acid tryptophan within the mitochondria. These differences reflect the ancient and semi-autonomous nature of mitochondria and necessitate a specialized set of tRNAs to accurately decode the organelle's genome.

**138.** (A) Inosine (I) : pairs with A, U, or C
Inosine is a modified base often found at the first (5') position of tRNA anticodons, where it plays a key role in wobble base-pairing. Due to its unique chemical structure, inosine can form hydrogen bonds with uracil, cytosine, or adenine at the third position of the codon. This versatility allows a single tRNA molecule to recognize three different synonymous codons, increasing the efficiency of translation.

**139.** (B) Downstream frame alteration and premature termination
A frameshift mutation, such as the insertion of a single nucleotide, alters the triplet reading frame of the mRNA from that point onward. This change typically results in the production of a completely different amino acid sequence downstream of the mutation. Furthermore, because the reading frame is shifted, the ribosome is likely to encounter a premature stop codon, leading to the synthesis of a truncated protein.

**140.** (A) I, II and IV
The genetic code is a triplet-based system that is nearly universal and highly degenerate. This degeneracy often involves synonymous mutations at the third codon position, which do not alter the protein sequence. Inosine in the anticodon allows for flexible wobble pairing with multiple bases. While most organisms follow the standard code, mitochondria have evolved specific deviations, meaning they do not follow the universal rules strictly.

**141.** (A) Covalently attaches the correct amino acid to its corresponding tRNA
An aminoacyl-tRNA synthetase is an essential enzyme that ensures the accuracy of the genetic code by "charging" tRNAs with their correct amino acids. Each synthetase is specific for one amino acid and its corresponding tRNAs. The enzyme catalyzes a two-step reaction that results in a covalent ester bond between the amino acid and the tRNA, providing the substrate for protein synthesis on the ribosome.

**142.** (C) Activation of the amino acid with ATP to form an aminoacyl-AMP intermediate
The first step in the aminoacylation of tRNA is the activation of the amino acid. The aminoacyl-tRNA synthetase binds both the amino acid and a molecule of ATP. It then catalyzes the formation of an aminoacyl-AMP intermediate, while releasing a molecule of pyrophosphate. This high-energy intermediate remains bound to the enzyme and provides the necessary energy for the subsequent transfer of the amino acid to tRNA.

**143.** (D) The 2'-hydroxyl group
Aminoacyl-tRNA synthetases are divided into two structural classes based on their active site architecture. Class I enzymes typically function as monomers and initially attach the activated amino acid to the 2' hydroxyl group of the terminal adenosine on the tRNA. Following this initial attachment, the amino acid may undergo a transesterification to the 3' position before it is utilized by the ribosome during protein synthesis.

**144.** (C) Double-sieve proofreading
The "double-sieve" mechanism is a proofreading strategy used by some aminoacyl-tRNA synthetases to ensure high fidelity. The first sieve is the activation site, which excludes amino acids that are larger than the correct one. The second sieve is a separate editing site that accommodates and hydrolyzes amino acids that are smaller or structurally similar to the correct one, preventing the formation of mischarged tRNA molecules.

**145.** (B) It accommodates only incorrect amino acids smaller than the correct one
In the double-sieve mechanism, the editing site of an aminoacyl-tRNA synthetase is specifically designed to recognize and hydrolyze incorrect aminoacyl-AMP intermediates or mischarged tRNAs. This site is physically sized to exclude the correct amino acid while allowing smaller, structurally similar, but incorrect amino acids to enter and be cleaved. This selective hydrolysis ensures that only the correctly charged tRNA is released for use in translation.

**146.** (D) Class II synthetases typically function as monomers and attach the amino acid to the 2'-OH
Class II aminoacyl-tRNA synthetases are structurally distinct from Class I enzymes. They

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protein. This process allows the same gene to produce two different proteins with distinct roles in lipid transport.

**129.** (B) Altered protein sequence affecting neuroreceptors
RNA editing mediated by ADAR enzymes can have significant biological effects by altering the amino acid sequence of proteins. A well-studied example occurs in the transcripts of various neuroreceptors, where A-to-I editing changes the properties of ion channels in the brain. This modification is essential for proper neurological function, and defects in this editing process have been linked to various diseases and behavioral abnormalities.

**130.** (D) I, II and III
RNA processing involves several sophisticated mechanisms. Group I introns require an external guanosine for splicing, while RNA editing in trypanosomes uses guide RNAs to insert or delete uridines. ADAR enzymes, which convert adenosine to inosine, specifically require double-stranded RNA structures for their activity. These diverse processes allow organisms to significantly expand and diversify their genetic information beyond the original DNA sequence.

**131.** (A) Degeneracy
The degeneracy of the genetic code is a fundamental property where multiple different codons can specify the same amino acid. This redundancy typically occurs at the third position of the codon. Degeneracy provides a buffer against the deleterious effects of mutations, as many single-base changes may not result in an amino acid substitution, thereby maintaining the integrity of the resulting protein's structure and function.

**132.** (C) Codons that specify the same amino acid
Synonymous codons are different triplet sequences that encode the same amino acid during translation. This phenomenon is the basis of the genetic code's degeneracy. For example, several codons might all signal the incorporation of leucine into a polypeptide chain. Although they result in the same protein sequence, synonymous codons can influence the speed of translation and the folding of the nascent protein.

**133.** (A) The third position (3' end)
The Wobble Hypothesis suggests that the base-pairing rules between the third position of an mRNA codon and the first position of a tRNA anticodon are less stringent. This flexibility allows a single tRNA to recognize and pair with more than one synonymous codon. This mechanism reduces the total number of different tRNA molecules required by a cell while still ensuring the accurate translation of the code.

**134.** (D) 5'-CAU-3'
The interaction between a codon and an anticodon is antiparallel. For an mRNA codon with the sequence 5'-AUG-3', the corresponding tRNA anticodon must be complementary and oriented in the opposite direction. Therefore, the anticodon sequence, when read in the standard 5' to 3' direction, is 5'-CAU-3'. This specific pairing ensures that the correct amino acid, in this case methionine, is delivered to the ribosome.

**135.** (B) Non-random usage of synonymous codons by organisms
Codon bias refers to the observation that organisms do not use synonymous codons with equal frequency. Even though multiple codons can specify the same amino acid, certain ones are preferred over others in highly expressed genes. This preference often correlates with the abundance of corresponding tRNA species in the cell, suggesting that codon bias is an evolutionary adaptation to optimize the efficiency and accuracy of translation.

**136.** (C) The code is universal, with no known exceptions in mitochondrial or viral genomes
While the genetic code is highly conserved and nearly universal across all known forms of life, it is not absolutely universal. Several exceptions have been identified, particularly in mitochondrial genomes and some ciliated protozoans. In these cases, specific codons may be reassigned to different amino acids or stop signals. These variations represent evolutionary diversions from the standard code while maintaining the basic triplet structure.

**137.** (D) UGA codes for Tryptophan instead of a stop signal
The human mitochondrial genetic code exhibits several notable deviations from the standard nuclear code. One significant variation is that the UGA codon, which normally serves as a

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**83.** (A) Tryptophan
The trpL leader sequence contains a small open reading frame that encodes a short peptide. This sequence is notable for having two consecutive codons for the amino acid tryptophan. The speed at which a ribosome translates these tandem codons is sensitive to the availability of charged tryptophanyl-tRNA, which serves as a sensor to regulate the transcription of the entire operon through attenuation.

**84.** (C) Ribosome stalling is lost, causing premature attenuation
The tandem tryptophan codons in the leader sequence are essential for the ribosome to stall during low tryptophan levels. If these codons are removed, the ribosome will not stall even when tryptophan is scarce. Instead, it will continue translating, which allows the formation of the terminator hairpin in the RNA. This leads to premature attenuation and reduced expression of the operon genes.

**85.** (A) The 3:4 terminator hairpin
When tryptophan levels are high, the ribosome quickly translates the tandem codons in the leader sequence and proceeds to cover region 2. This prevents region 2 from pairing with region 3. Consequently, region 3 base-pairs with region 4 to form the 3:4 terminator hairpin. This structure acts as a signal for RNA polymerase to stop transcription before it reaches the structural genes.

**86.** (D) Attenuation is a mechanism of positive control that requires cAMP
Transcription attenuation in the trp operon is a mechanism that relies on the physical coupling of transcription and translation. It is not a form of positive control and does not involve the cAMP-CAP system used in catabolite repression. Instead, it serves as a fine-tuning mechanism that responds directly to the levels of charged tRNA in the cell to regulate the synthesis of tryptophan.

**87.** (B) Ribosome-mediated transcription attenuation
The trp operon utilizes a unique regulatory mechanism called attenuation, which is not found in the lac operon. Attenuation allows the cell to respond to the levels of charged tryptophanyl-tRNA by sensing the speed of a ribosome on a leader transcript. This process involves the formation of alternative RNA secondary structures that determine whether transcription will continue into the structural genes or terminate prematurely.

**88.** (C) Antiterminator loop : formed by base pairing between regions 2 and 3
In the trp operon, the formation of the 2:3 antiterminator hairpin is a critical event that allows transcription to continue. This structure forms when the ribosome stalls at the tandem tryptophan codons during amino acid starvation, leaving region 2 free to pair with region 3. This prevent the formation of the 3:4 terminator hairpin, ensuring that the structural genes are fully transcribed.

**89.** (D) Depletion of charged tryptophanyl-tRNA during starvation
Ribosome stalling during the attenuation of the trp operon is caused by the lack of charged tryptophanyl-tRNA. When the cell is starved for tryptophan, the ribosome cannot find the necessary tRNA to translate the tandem tryptophan codons in the leader sequence. This pause in translation allows specific regions of the nascent RNA to base-pair and form an antiterminator structure, promoting full transcription.

**90.** (C) I, II and III
The trp operon is a repressible system where the apo-repressor requires tryptophan to bind the operator. This provides a coarse level of control. Attenuation offers an additional layer of regulation that is sensitive to the availability of charged tRNAs. Together, these mechanisms ensure that the biosynthetic enzymes are only produced when the cell's supply of tryptophan is insufficient for its needs.

**91.** (B) 7-methylguanosine
The 5' cap is a critical modification added to eukaryotic pre-mRNA shortly after the initiation of transcription. It consists of a 7-methylguanosine residue. This modified nucleotide is attached to the first base of the transcript in a unique orientation. The cap plays essential roles in protecting the RNA from degradation, facilitating nuclear export, and promoting the initiation of translation in the cytoplasm.

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generally function as dimers or tetramers and possess a characteristic active site composed of β-sheets. Unlike Class I enzymes, Class II synthetases typically attach the amino acid directly to the 3' hydroxyl group of the terminal adenosine of the tRNA. This classification highlights the diverse evolutionary strategies used to achieve accurate tRNA charging.

**147.** (A) An ester bond between the amino acid carboxyl group and the ribose of the terminal adenine
During the transfer step of the aminoacylation reaction, the amino acid is moved from the aminoacyl-AMP intermediate to the tRNA. This process results in the formation of a high-energy ester bond between the carboxyl group of the amino acid and a hydroxyl group on the terminal ribose of the tRNA. This ester bond preserves much of the energy from the ATP used during activation.

**148.** (C) Activation step : releases pyrophosphate (PPi)
The activation of an amino acid by its specific tRNA synthetase is the energy-requiring first step of the charging process. During this reaction, the enzyme uses ATP to create an aminoacyl-AMP intermediate, which results in the release of inorganic pyrophosphate. This step is crucial because the subsequent cleavage of pyrophosphate by pyrophosphatase makes the overall reaction thermodynamically favorable, driving the formation of the charged tRNA.

**149.** (A) The ribosome incorporates whatever amino acid is attached
The ribosome lacks the ability to verify if the amino acid attached to a tRNA is correct; it only checks the codon-anticodon match. Therefore, the accuracy of translation depends entirely on the aminoacyl-tRNA synthetases. If a synthetase attaches the wrong amino acid, that incorrect residue will be incorporated into the protein, potentially leading to misfolding or loss of function, which can be deleterious.

**150.** (C) IV and V
Aminoacyl-tRNA synthetases are complex enzymes that use ATP, not GTP, to activate amino acids. Class II synthetases have a specific structure and typically bind to the major groove, not the minor groove, of the tRNA acceptor stem. These errors in understanding reflect common misconceptions about the metabolic requirements and structural interactions involved in the essential process of charging tRNA molecules for protein synthesis.

**151.** (B) Sedimentation rate in an ultracentrifuge, influenced by mass, shape, and density
The Svedberg unit is a non-metric unit that characterizes the sedimentation rate of a particle, such as a ribosome or an organelle, during ultracentrifugation. This value is determined not only by the mass of the particle but also by its density and overall shape. Consequently, Svedberg values are not additive; for example, the combination of two subunits results in a different total S value.

**152.** (C) 30S and 50S
The prokaryotic 70S ribosome is a large ribonucleoprotein complex composed of two unequal subunits. The small subunit, designated as 30S, is responsible for mRNA binding and decoding. The large subunit, designated as 50S, contains the peptidyl transferase center where peptide bonds are formed. Together, these subunits coordinate the complex series of events required for the accurate and efficient translation of genetic information.

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**153.** (D) Binding the incoming aminoacyl-tRNA for decoding
The aminoacyl (A) site is one of the three functional binding sites for tRNA on the ribosome. Its primary role is to accommodate the incoming aminoacyl-tRNA that matches the current codon of the mRNA. The binding at this site is the first step in each elongation cycle, where the fidelity of translation is checked through the interaction between the codon and the anticodon.

**154.** (A) The 23S rRNA
The peptidyl transferase center is the catalytic heart of the ribosome, responsible for forming peptide bonds between amino acids. In prokaryotes, this activity is located within the 23S rRNA of the large 50S subunit. Because this site consists entirely of RNA, antibiotics that block this center target the ribosomal RNA itself, highlighting the essential role of RNA in the catalytic process of translation.

hexamer is located about ten to thirty nucleotides upstream of the cleavage site. It is recognized by specific proteins that coordinate the recruitment of an endonuclease to cleave the transcript and poly(A) polymerase to add the adenine tail to the new 3' end.

**102.** (D) It adds adenines in a template-independent manner
Poly(A) polymerase is the enzyme responsible for the synthesis of the poly(A) tail at the 3' end of eukaryotic mRNA. Unlike most polymerases, it does not require a DNA or RNA template to guide nucleotide addition. Instead, it adds adenine residues in a template-independent manner, using ATP as a substrate. This tail is essential for mRNA stability, nuclear export, and translation.

**103.** (B) The CPSF specificity factor
The cleavage and polyadenylation specificity factor is a multi-protein complex that plays a central role in 3' end processing. It specifically binds to the AAUAAA signal sequence on the nascent pre-mRNA. This binding event is crucial for defining the site of cleavage and for recruiting other necessary factors, including the endonuclease and poly(A) polymerase, to ensure the correct formation of the tail.

**104.** (C) CstF binding fails, reducing cleavage efficiency
The G/U-rich element is a regulatory sequence located downstream of the cleavage site in eukaryotic pre-mRNAs. It is recognized and bound by the cleavage stimulation factor. If this element is mutated, the binding of CstF is impaired, which significantly reduces the efficiency of the cleavage process. Consequently, the proper polyadenylation of the mRNA may fail, affecting its eventual stability and translation.

**105.** (D) Enhanced stability and translation initiation
The addition of a poly(A) tail to the 3' end of an mRNA molecule has profound effects on its biological function. It provides a binding site for poly(A) binding proteins, which protect the transcript from degradation by 3' exonucleases. Furthermore, the tail interacts with the 5' cap-binding complex to promote efficient translation initiation and facilitates the export of the mRNA.

**106.** (A) Cleavage of the pre-mRNA occurs upstream of the AAUAAA sequence
In the processing of eukaryotic pre-mRNA, the cleavage event occurs downstream of the AAUAAA consensus sequence, typically between ten and thirty nucleotides away. This cleavage creates the free 3' hydroxyl group that serves as the primer for the template-independent addition of adenine residues by poly(A) polymerase. Therefore, the statement that cleavage occurs upstream of the AAUAAA sequence is factually incorrect.

**107.** (B) It involves the addition of nucleotides without an RNA or DNA template
Polyadenylation is a unique RNA processing step because it involves the addition of a long string of adenine nucleotides without the use of a DNA or RNA template. This template-independent synthesis is carried out by poly(A) polymerase after the transcript has been cleaved. This process distinguishes it from both transcription and other processing events like capping, which do not involve template-free polymerization.

**108.** (B) PAP : requires ATP as a substrate
Poly(A) polymerase is the enzyme that catalyzes the formation of the poly(A) tail at the 3' end of mRNA. This reaction requires ATP as a substrate to provide the adenine residues that are added to the transcript. PAP works in coordination with other factors that identify the polyadenylation signal and cleave the pre-mRNA, ensuring that the tail is added at the correct location.

**109.** (A) PABP interaction with the eIF4G subunit
The circularization of eukaryotic mRNA is facilitated by a physical bridge between the 5' and 3' ends. Poly(A) binding proteins, which are bound to the poly(A) tail, interact directly with the eIF4G subunit of the cap-binding complex. This interaction brings the two ends of the mRNA into close proximity, which enhances translation efficiency by promoting the recycling of ribosomes and stabilizing the transcript.

**110.** (C) A is true but R is false.
Polyadenylation is an essential step for the translation and stability of most eukaryotic mRNAs. However, it is not required for the assembly of the spliceosome or the removal of introns. Splicing is a distinct process that typically occurs co-transcriptionally, often before the 3' end of the pre-mRNA has been cleaved

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**164.** (C) Premature association of the 30S and 50S subunits before mRNA binding
Initiation Factor 3 is a critical protein in prokaryotic translation that binds to the small 30S subunit. Its primary role is to prevent the large 50S subunit from prematurely associating with the 30S subunit before the mRNA and initiator tRNA are properly bound. If IF3 is defective, the ribosomal subunits may join incorrectly, leading to a failure in the initiation process.

**165.** (A) IF2 release and stable joining of the large 50S subunit
Initiation Factor 2 is a GTP-binding protein that escorts the initiator tRNA to the 30S ribosomal subunit. Once the start codon is recognized and the initiator tRNA is correctly positioned in the P site, the hydrolysis of the GTP bound to IF2 is triggered. This event leads to the release of IF2 and other initiation factors, allowing the large 50S subunit to join.

**166.** (D) The initiator tRNA binds to the A site of the small subunit
During the initiation of translation in prokaryotes, the initiator tRNA does not bind to the A site. Instead, it is specifically directed to the P site of the small ribosomal subunit by initiation factors. The A site is kept empty by the presence of $IF_1$, ensuring that the first peptide bond can only be formed after an aminoacyl-tRNA enters the A site during elongation.

**167.** (B) It acts as a GTPase that escorts the initiator tRNA to the P site
IF2 is a specialized GTPase that plays a vital role in prokaryotic translation initiation. Its main function is to recognize the formylated methionine on the initiator tRNA and deliver this tRNA specifically to the P site of the 30S ribosomal subunit. The energy provided by GTP hydrolysis later assists in the docking of the 50S subunit to form the functional 70S ribosome.

**168.** (C) Shine-Dalgarno sequence : base pairs with the 3' end of 16S rRNA
In prokaryotes, the Shine-Dalgarno sequence on the mRNA is essential for correct ribosome positioning. It functions by base-pairing with a complementary sequence located at the 3' end of the 16S rRNA within the 30S subunit. This interaction ensures that the start codon is placed directly in the P site, allowing for the accurate and efficient initiation of protein synthesis on the messenger RNA.

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**169.** (D) Independent Shine-Dalgarno sequences for each cistron
Bacterial mRNAs are often polycistronic, meaning they contain multiple independent coding regions for different proteins. Each of these coding regions is preceded by its own Shine-Dalgarno sequence. This allows the ribosome to initiate translation independently at each start site along the single mRNA molecule. This arrangement ensures that all the proteins in an operon can be synthesized efficiently from one transcript.

**170.** (A) P-2, Q-1, R-3, S-4
Prokaryotic translation initiation is a coordinated process involving three main factors. $IF_1$ blocks the A site, while IF3 prevents the premature association of the large and small subunits. IF2 is a GTP-binding protein that ensures the initiator tRNA, carrying formylmethionine, is correctly placed into the P site. Together, these components ensure that the ribosome is accurately assembled and positioned to begin synthesis.

**171.** (B) The 5' m7G cap
In eukaryotes, the initiation of translation typically follows a cap-dependent scanning mechanism. The 40S ribosomal subunit, associated with several initiation factors, first binds to the 7-methylguanosine cap at the 5' end of the mRNA. From there, the subunit moves along the 5' untranslated region in a 5' to 3' direction, scanning for the appropriate start codon to begin the synthesis of the protein.

**172.** (B) Optimized AUG context enhancing translation initiation
The Kozak consensus sequence is a specific nucleotide pattern that surrounds the start codon in eukaryotic mRNAs. This sequence, typically represented as (gcc)gccRccAUGG, plays a critical role in the efficiency of translation initiation. It helps the scanning 40S ribosomal subunit to correctly identify the optimal AUG start codon, ensuring that the protein is synthesized in the correct reading frame and at high levels.

**173.** (A) eIF4E
The eIF4F complex is a central player in eukaryotic translation initiation. Within this

site. This reaction releases the new protein from the ribosome.

**246.** (D) Ubiquitination targets proteins for degradation within the lysosome
Ubiquitination is a post-translational modification that typically targets proteins for degradation by the 26S proteasome in the cytoplasm, not within the lysosome. In this process, multiple ubiquitin molecules are covalently attached to the target protein, serving as a specific recognition signal for the proteasome. The proteasome then unfolds and breaks down the protein into small peptides, which is essential for cellular quality control.

**247.** (B) Translational control using existing mRNA
Translational control offers a faster response to environmental changes than transcriptional control because it acts on mRNA molecules that are already synthesized and present in the cytoplasm. By regulating the activity of existing transcripts, the cell can immediately increase or decrease the production of specific proteins without waiting for the multi-step processes of gene transcription, RNA processing, and nuclear export to occur.

**248.** (C) Pre-mRNA : Addition of a 3' poly(A) tail
In eukaryotic cells, the 3' end of a pre-mRNA molecule is processed through the addition of a poly(A) tail. This modification occurs after the transcript has been cleaved at a specific site directed by consensus sequences. The poly(A) tail is essential for several functions, including protecting the mRNA from degradation by 3' exonucleases, facilitating its export from the nucleus, and enhancing its translation.

**249.** (D) Non-standard bonding at the 5' anticodon
The "wobble" effect is caused by the flexible base-pairing that occurs at the third position of a codon and the first position of an anticodon. This non-standard pairing allows certain tRNA molecules to recognize more than one synonymous codon. For example, a single tRNA might be able to pair with codons ending in different nucleotides, which reduces the total number of tRNAs required.

**250.** (A) I and III
Protein synthesis is a highly regulated process where folding often begins co-translationally as the polypeptide emerges from the ribosome. To maintain cellular health, misfolded proteins are recognized by quality control systems and tagged with polyubiquitin for degradation by the proteasome. Other modifications, like phosphorylation, are reversible and allow for the dynamic control of protein activity. Together, these processes ensure that proteins are functional.

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tRNA to the ribosomal A site. Upon correct codon-anticodon pairing, EF-Tu hydrolyzes GTP and dissociates from the ribosome, allowing the amino acid to participate in synthesis.

**183.** (B) Nucleophilic attack
Peptide bond formation is a nucleophilic attack that occurs during translation elongation. The amino group of the aminoacyl-tRNA in the A site acts as a nucleophile, attacking the ester bond that links the growing polypeptide chain to the tRNA in the P site. This reaction, catalyzed by the peptidyl transferase center, results in the transfer of the entire polypeptide to the tRNA in the A site.

**184.** (C) The aminoacyl-tRNA cannot be released from EF-Tu into the A site, halting elongation
The hydrolysis of GTP by EF-Tu is a critical control point in translation. This event only occurs after successful codon-anticodon recognition in the A site. If EF-Tu cannot hydrolyze its GTP, it will remain bound to the aminoacyl-tRNA and the ribosome. This failure prevents the release of the tRNA into the active site, thereby halting the elongation process and stopping protein synthesis.

**185.** (D) EF-G (in prokaryotes) or eEF2 (in eukaryotes)
Translocation is the step in translation where the ribosome moves one codon forward along the mRNA. This movement is catalyzed by an elongation factor, known as EF-G in prokaryotes or eEF2 in eukaryotes. These factors use the energy from GTP hydrolysis to drive the physical translocation of the tRNAs and the mRNA, shifting the peptidyl-tRNA from the A site to the P site.

**186.** (A) Translocation shifts the deacylated tRNA from the P site to the A site
Translocation is a critical step in translation elongation that involves the simultaneous movement of mRNA and tRNAs within the ribosome. During this process, the deacylated tRNA that was in the P site is moved to the E (exit) site, while the peptidyl-tRNA moves from the A site to the P site. This action clears the A site for the entry of the next aminoacyl-tRNA.

**187.** (B) eEF2
In eukaryotic translation, the factor eEF2 performs the role equivalent to the bacterial EF-G. It is a GTPase that facilitates the translocation of the ribosome along the mRNA after each peptide bond is formed. By hydrolyzing GTP, eEF2 provides the mechanical energy required to shift the tRNAs between the ribosomal sites and move the mRNA by one codon, ensuring continuous elongation.

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**188.** (B) EF-G : structurally mimics an aminoacyl-tRNA-EF-Tu complex to force translocation
EF-G is an elongation factor that plays a key role in the translocation step of protein synthesis. It is remarkable because its three-dimensional structure closely mimics that of the ternary complex formed by EF-Tu, GTP, and aminoacyl-tRNA. This structural mimicry allows EF-G to bind to the ribosomal A site, where it uses the energy from GTP hydrolysis to push the tRNAs into the next sites.

**189.** (A) The rotation of the small subunit relative to the large subunit (ratcheting)
Translocation requires a complex mechanical movement of the ribosomal subunits relative to one another. This movement, often called ratcheting, involves the rotation of the small subunit against the large subunit. This rotation is coupled with the action of elongation factors and GTP hydrolysis, facilitating the precise shifting of the mRNA and tRNAs through the ribosome's binding sites during each cycle of protein synthesis.

**190.** (A) II, IV, I, III
A single cycle of translation elongation consists of a repeating series of four steps. First, the correct aminoacyl-tRNA binds to the A site. Second, a peptide bond is formed, transferring the polypeptide to the A site. Third, the ribosome translocates, moving the tRNAs and mRNA. Finally, the uncharged tRNA is released from the E site, clearing the ribosome for the next cycle.

**191.** (D) It signals the termination of the polypeptide chain
Nonsense codons, also known as stop codons, do not specify an amino acid. Instead, they serve as essential signals for the termination of translation. When a ribosome encounters one of these codons (UAA, UAG, or UGA) in the A site, it does not recruit a tRNA. Instead, it binds specialized proteins called release factors, which

complex is a large channel that allows for the passage of fully folded proteins, often in complex with importin receptors. In contrast, the ER translocon is a narrow channel that requires the protein to be in an unfolded, linear polypeptide form.

**228.** (C) Sec translocon : translocates proteins across the ER membrane
The Sec61 translocon is the central protein-conducting channel in the endoplasmic reticulum membrane. It is responsible for the translocation of nascent polypeptides into the ER lumen or their insertion into the membrane during co-translational targeting. This complex works in close coordination with the SRP receptor to ensure that proteins with ER signal sequences are correctly directed to their appropriate cellular destination.

**229.** (A) Establish a gradient directing transport flow
The transport of proteins into and out of the nucleus is driven by a concentration gradient of the Ran GTPase in its two states. Ran-GTP is found in high concentrations within the nucleus, while Ran-GDP is more abundant in the cytoplasm. This gradient provides the directionality and energy for the transport process by regulating the binding and release of cargo from nuclear receptors.

**230.** (A) SRP binds signal sequence → Translation pauses → SRP binds receptor → Ribosome transferred to translocon → Translation resumes
Co-translational targeting to the ER involves a series of highly coordinated steps. First, the SRP binds to the emerging signal sequence, causing translation to pause. The SRP then binds to its receptor on the ER membrane, directing the ribosome to the translocon. Once the ribosome is transferred, the SRP is released, and translation resumes as the polypeptide is threaded into the lumen.

**231.** (B) eIF2
Global translational control allows a cell to rapidly adjust the overall rate of protein synthesis in response to stress or environmental changes. This regulation frequently targets the initiation phase, specifically by modifying the eukaryotic initiation factor eIF2. By altering the activity of this central factor, the cell can simultaneously decrease the translation of almost all mRNAs, conserving energy and resources during difficult conditions.

**232.** (C) Inhibition of the exchange factor eIF2B
Phosphorylation of the $\alpha$ subunit of eIF2 is a classic mechanism for inhibiting global translation. When eIF2 is phosphorylated, it binds with high affinity to its guanine nucleotide exchange factor, eIF2B, and acts as a competitive inhibitor. This prevents eIF2B from exchanging GDP for GTP on other eIF2 molecules, leading to a depletion of the active eIF2-GTP complex and halting translation.

**233.** (D) IRP protein blocking PIC scanning
The translation of ferritin mRNA is regulated by iron levels through an Iron Response Element in its 5' untranslated region. When cellular iron is low, an Iron Regulatory Protein binds to the IRE. This binding creates a physical block that prevents the 40S ribosomal subunit from scanning toward the start codon. This mechanism ensures that ferritin is not produced when iron is scarce.

**234.** (A) Rapid nuclease degradation from lack of binding
The transferrin receptor mRNA is regulated by iron levels through several Iron Response Elements located in its 3' untranslated region. In low iron conditions, Iron Regulatory Proteins bind to these IREs, which stabilizes the mRNA and protects it from degradation by nucleases. This leads to increased translation. If these elements are mutated, the protection is lost, and the mRNA is rapidly degraded.

**235.** (B) Deadenylation (shortening of the tail)
The primary pathway for mRNA degradation in eukaryotes begins with the gradual shortening of the poly(A) tail, a process known as deadenylation. This is carried out by specialized enzymes called deadenylases. Once the tail reaches a critically short length, the mRNA is no longer protected by poly(A) binding proteins, which triggers subsequent steps like decapping and rapid exonucleolytic digestion of the transcript.

**236.** (B) Decapping enzymes remove the 5' cap only after the poly(A) tail is significantly lengthened
The degradation of eukaryotic mRNA is a highly regulated process. Deadenylation, the shortening of the poly(A) tail, is the initial step that makes the transcript vulnerable.

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**201.** (D) After the synthesis of the polypeptide chain is completed
A post-translational modification refers to a chemical change that occurs to a protein after its synthesis on the ribosome has been completed. These modifications can include the addition of functional groups, such as phosphate or methyl groups, or the cleavage of the polypeptide chain. These changes are essential for regulating protein activity, stability, localization, and interactions within the complex environment of the cell.

**202.** (A) Asparagine N-linked glycosylation
N-linked glycosylation is a common post-translational modification where a carbohydrate chain is covalently attached to the nitrogen atom of an asparagine residue's side chain. This process typically occurs within the consensus sequence Asn-X-Ser/Thr. It is a critical modification for many secreted and membrane-bound proteins, playing a vital role in protein folding, stability, and cell-cell signaling within the endoplasmic reticulum and Golgi.

**203.** (B) Phosphatases
The regulation of protein function through phosphorylation is a dynamic and reversible process. While protein kinases are responsible for adding phosphate groups to specific residues, another class of enzymes called phosphatases is responsible for removing them. This balanced action of kinases and phosphatases allows the cell to rapidly toggle the activity of proteins on and off in response to various internal and external signals.

**204.** (B) Degradation via the 26S proteasome
Ubiquitination is a process where a small protein called ubiquitin is attached to a target protein. When a chain of ubiquitin molecules is linked specifically through their Lysine-48 residues, it serves as a powerful signal for the cell. This polyubiquitin tag is recognized by the 26S proteasome, which then captures and degrades the target protein into small peptides, effectively removing it from the cell.

**205.** (A) Enzymatic cleavage of peptide bonds
Proteolytic processing is a form of post-translational modification that involves the precise cleavage of peptide bonds by enzymes called proteases. This process is often used to convert an inactive precursor protein, such as a proenzyme or a prohormone, into its active functional form. A classic example is the maturation of insulin, where a precursor molecule is cleaved to produce the final active hormone.

**206.** (C) Acetylation of histones generally correlates with transcriptional activation
Histone acetylation is a post-translational modification that typically correlates with the activation of transcription rather than its repression. Enzymes called histone acetyltransferases add acetyl groups to the tails of histones, which neutralizes their positive charges and relaxes the chromatin structure. This change increases the accessibility of DNA to the transcription machinery, facilitating gene expression. Therefore, the statement that it causes repression is incorrect.

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**207.** (D) In the endoplasmic reticulum (ER)
N-linked glycosylation is a sophisticated process that begins co-translationally in the endoplasmic reticulum. As the nascent polypeptide is being synthesized and translocated into the ER lumen, a pre-assembled core oligosaccharide is transferred to specific asparagine residues. This initial modification is essential for proper protein folding and serves as a foundation for further carbohydrate processing that occurs later as the protein moves.

**208.** (C) Lipidation : anchors proteins to biological membranes
Lipidation is a post-translational modification where lipid groups, such as fatty acids or prenyl groups, are covalently attached to a protein. This modification increases the protein's hydrophobicity, allowing it to become anchored to biological membranes. This is essential for the proper localization and function of many signaling proteins, as it ensures they are positioned correctly to interact with other membrane-bound components.

**209.** (B) Specific target recognition and transfer
The ubiquitination cascade relies on a sequence of three enzymes (E1, E2, and E3) to tag proteins for degradation by the proteasome. The E3 ubiquitin ligase functions as the crucial final component by providing substrate specificity, directly recognizing and binding to the specific target protein. It then facilitates the transfer of the ubiquitin molecule from the E2 ubiquitin-conjugating enzyme to the target, ensuring

that only the correct proteins are marked for destruction.

**210.** (A) Both A and R are true and R is the correct explanation of A.
Proteolytic processing is fundamentally an irreversible modification because it involves the breakage of strong covalent peptide bonds. Once a portion of the polypeptide chain has been removed by a protease, there are no standard cellular mechanisms capable of re-ligating the fragments back into the original precursor form. This irreversibility is important for ensuring that biological processes, like enzyme activation or hormone maturation, proceed.

**211.** (A) Binding hydrophobic regions to prevent aggregation
Molecular chaperones of the Hsp70 class play a vital role in protein folding by binding to exposed hydrophobic patches on nascent or denatured polypeptides. This binding prevents the hydrophobic regions from interacting inappropriately with other proteins, thereby inhibiting the formation of non-functional aggregates. By stabilizing these intermediate states, Hsp70 allows the protein the opportunity to fold correctly into its native, functional conformation.

**212.** (C) Chaperonins
Chaperonins are a specialized class of molecular chaperones that form large, barrel-shaped complexes. A prime example is the GroEL-GroES system found in bacteria. These complexes provide a unique, isolated environment where an unfolded or misfolded protein can fold into its native state without the risk of aggregating with other proteins in the crowded cytoplasm. This sequestered folding is essential for many proteins.

**213.** (A) ATP hydrolysis for conformational cycles
The GroEL-GroES complex utilizes the energy from ATP hydrolysis to drive its functional cycle. ATP binding to the GroEL rings triggers conformational changes that allow the GroES lid to attach, creating an enclosed folding chamber. The subsequent hydrolysis of ATP and the binding of new ATP molecules coordinate the release of the GroES lid and the folded protein, ensuring the cycle continues.

**214.** (D) The chamber lid cannot seal the environment
GroES is a critical co-chaperonin that serves as the "lid" for the GroEL barrel during the protein folding cycle. Its primary role is to seal the chamber after an unfolded protein has entered. This creates a protected, hydrophilic environment where the protein can fold in isolation. If GroES is missing, the chamber cannot be closed, and the necessary environment for sequestered folding cannot be established.

**215.** (B) The ubiquitin-proteasome system
The cell maintains a rigorous quality control system to manage misfolded proteins. When a protein cannot be folded correctly by chaperones, it is often recognized by specific E3 ubiquitin ligases. These enzymes tag the defective protein with a polyubiquitin chain, which serves as a signal for the ubiquitin-proteasome system. This pathway ensures that potentially toxic misfolded proteins are efficiently degraded and removed.

**216.** (C) The 26S proteasome degrades proteins into small peptide fragments through multiple cleavage sites
The 26S proteasome is a complex molecular machine designed to degrade polyubiquitinated proteins. However, it does not simply cleave them at multiple random sites. Instead, the 19S regulatory particle first recognizes the ubiquitin tag and unfolds the protein using ATP. The protein is then translocated into the 20S core particle, where specific proteolytic active sites cleave it into small peptides in a controlled manner.

**217.** (D) It provides a physically enclosed cage or chamber for the entire protein to fold
While both Hsp70 and GroEL are molecular chaperones, GroEL belongs to the chaperonin class and features a unique mechanism. Unlike Hsp70, which binds to hydrophobic segments of a protein, GroEL forms a large, double-ring structure that provides a physically enclosed cage. This chamber allows an entire unfolded protein to fold in an isolated environment, away from the crowded cytoplasm, which prevents aggregation.

**218.** (A) GroES : acts as the "lid" for the GroEL complex
GroES is a essential component of the bacterial chaperonin system, acting as a lid that covers the opening of the GroEL barrel. This interaction

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is regulated by ATP and is necessary to create a closed environment where protein folding can occur. The binding of GroES also induces conformational changes in GroEL that expand the inner chamber, facilitating the folding of the sequestered substrate.

**219.** (B) Prolonged exposure of buried hydrophobic patches
The quality control machinery of the cell recognizes misfolded proteins primarily by detecting the prolonged exposure of hydrophobic patches. In a correctly folded protein, these hydrophobic regions are buried in the core. Their presence on the surface serves as a universal signal that the protein is defective. Specific E3 ubiquitin ligases bind to these exposed regions and tag the protein for degradation.

**220.** (A) P-2, Q-4, R-3, S-1
The cellular machinery for protein folding and degradation is highly specialized. Hsp70 and GroEL act as chaperones that assist in folding, with GroEL providing a unique chamber. Ubiquitin is a small protein that serves as a tag, while the 26S proteasome is the complex responsible for degrading these tagged proteins. These components work together to ensure that only correctly folded proteins remain active.

**221.** (A) Endoplasmic reticulum (ER)
The Signal Hypothesis explains the fundamental mechanism by which proteins are targeted to the endoplasmic reticulum. It proposes that proteins destined for secretion or membrane insertion contain a specific signal sequence. This sequence is recognized by the cellular machinery as it emerges from the ribosome, directing the entire ribosome-polypeptide complex to the ER membrane, where the protein is co-translationally translocated into the lumen.

**222.** (C) 6-12 hydrophobic residues near the N-terminus
An ER-targeting signal sequence is a short stretch of amino acids typically found at the N-terminus of a nascent protein. Its most defining feature is a core region of six to twelve hydrophobic residues. This hydrophobic core is essential for recognition by the signal recognition particle, which then facilitates the delivery of the ribosome and the growing polypeptide to the ER translocon.

**223.** (D) Signal Recognition Particle
The Signal Recognition Particle is a ribonucleoprotein complex that plays a central role in co-translational protein targeting. As soon as an ER signal sequence emerges from the ribosome, the SRP binds to it. this binding event causes a temporary pause in translation, which prevents the protein from folding in the cytoplasm and ensures it can be correctly translocated into the endoplasmic reticulum.

**224.** (C) Stalled transfer from SRP to the translocon
The successful transfer of the ribosome-nascent chain complex from the SRP to the ER translocon is a highly regulated process. Both the SRP and its receptor are GTPases, and their interaction and subsequent dissociation depend on GTP binding and hydrolysis. If the GTPase activity of the receptor is inhibited, the SRP cannot be released properly, which blocks the transfer of the polypeptide.

**225.** (B) Unfolded, stabilized by chaperones like Hsp70
Unlike ER targeting, protein import into mitochondria is typically a post-translational process. Proteins destined for the mitochondria are fully synthesized in the cytoplasm. To pass through the narrow translocation channels in the mitochondrial membranes, these proteins must be maintained in an unfolded, linear state. This is achieved by the binding of molecular chaperones, such as Hsp70, which prevent premature folding and aggregation.

**226.** (D) Nuclear localization signals (NLS) are typically not cleaved off after import
Nuclear localization signals are specialized sequences that direct proteins from the cytoplasm into the nucleus through the nuclear pore complex. Unlike the signal sequences used for ER or mitochondrial targeting, an NLS is usually not cleaved off once the protein has reached its destination. This allows the protein to be re-imported into the nucleus should it leak out or following nuclear envelope reassembly.

**227.** (A) Nuclear import uses a protein channel (NPC) that accommodates fully folded proteins; ER import typically requires unfolded linear chains passing through the translocon
A fundamental difference between nuclear and ER protein import lies in the state of the protein during translocation. The nuclear pore

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initiate the process of releasing the completed polypeptide chain.

**192.** (C) $RF_1$
In prokaryotes, two primary release factors are responsible for recognizing stop codons. $RF_1$ specifically identifies and binds to the UAA and UAG codons. Its role is to mimic the structure of a tRNA and enter the ribosomal A site, where it triggers the hydrolysis of the bond between the finished polypeptide and the tRNA in the P site, leading to the termination of translation.

**193.** (B) A water molecule
Translation termination involves a shift in the catalytic activity of the ribosome's peptidyl transferase center. When a release factor binds to the A site, it positions itself to interact with the active site. Instead of facilitating the attack of an amino group, it promotes the attack of a water molecule on the ester bond of the peptidyl-tRNA, resulting in the release of the protein.

**194.** (D) Stalled 70S ribosome accumulation after termination
The Ribosome Recycling Factor is essential for the final stage of protein synthesis in bacteria. After the polypeptide has been released, the ribosome remains bound to the mRNA and contains a deacylated tRNA. RRF, working with EF-G, uses the energy from GTP hydrolysis to disassemble this complex. If RRF is absent, stalled ribosomes accumulate, preventing them from being reused for new translation events.

**195.** (A) It recognizes all three stop codons (UAA, UAG, UGA) on its own
Eukaryotic translation termination is more streamlined than in prokaryotes, as it primarily relies on a single class I release factor called $eRF_1$. This protein is remarkable because it has the ability to recognize all three stop codons: UAA, UAG, and UGA. By binding to any of these signals in the A site, $eRF_1$ initiates the hydrolysis of the peptidyl-tRNA bond and the release of the protein.

**196.** (C) Eukaryotes require three distinct release factors ($eRF_1$, eRF2, eRF3) to recognize the three stop codons
Translation termination in eukaryotes does not require three different class I release factors. Unlike prokaryotes, which use $RF_1$ and RF2 for different codons, eukaryotes utilize a single factor, $eRF_1$, to recognize all three stop codons. This protein works alongside other factors like eRF3, a GTPase, to ensure the efficient release of the completed polypeptide and the subsequent disassembly of the translation machinery for recycling.

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**197.** (A) It acts as a GTPase that stimulates the termination process and factor release
In eukaryotes, the factor eRF3 plays a role similar to the prokaryotic RF3. It is a GTPase that forms a complex with the class I release factor $eRF_1$. The hydrolysis of GTP by eRF3 provides the energy and conformational changes needed to stimulate the termination process and facilitate the release of the factors from the ribosome after the polypeptide chain has been successfully cleaved.

**198.** (D) RF2 : recognizes UAA and UGA
In the prokaryotic system of translation termination, the two class I release factors have specific codon preferences. RF2 is responsible for recognizing the UAA and UGA stop codons. When it enters the ribosomal A site, it triggers the release of the finished protein. This specificity ensures that all possible stop signals are accurately detected by the cell's machinery to maintain the correct protein length.

**199.** (B) Subunit separation via GTP hydrolysis with RRF
EF-G, a well-known elongation factor, also plays a critical role in the ribosome recycling phase in bacteria. After the release of the completed polypeptide, EF-G works in conjunction with the Ribosome Recycling Factor. Using the energy from GTP hydrolysis, EF-G helps to disassemble the remaining post-termination complex, consisting of the ribosome, mRNA, and deacylated tRNA, so the subunits can be reused.

**200.** (A) I, II, III and IV
Translation termination and recycling are complex processes involving multiple factors. $RF_1$ and RF2 recognize specific stop codons, with both recognizing UAA. During termination, the peptidyl transferase activity is converted to an esterase activity to release the protein. Following this, the ribosome is disassembled, and IF3 binds to the small subunit to prevent premature reassociation, ensuring the machinery is ready for a new initiation.

Following this, decapping enzymes remove the 5' cap. This removal only occurs after the tail has been shortened, not lengthened. Once decapped, the mRNA is quickly degraded by 5' to 3' exonucleases, ensuring efficient clearance of transcripts.

**237.** (A) 5'-to-3' involves Dcp1/Dcp2 and Xrn1 activity
Eukaryotic mRNA degradation can proceed via two main routes following deadenylation. The 5'-to-3' pathway involves the removal of the 5' cap by the Dcp1/Dcp2 complex, followed by digestion of the RNA by the Xrn1 exonuclease. In contrast, the 3'-to-5' pathway involves the progressive degradation of the transcript from its 3' end by the exosome, a large multi-protein complex with exonucleolytic activity.

**238.** (C) Ferritin IRE regulation : controls translation initiation based on iron levels
Ferritin mRNA regulation is a classic example of translational control that responds to cellular nutrient levels. By using an Iron Response Element in the 5' UTR, the cell can specifically inhibit the initiation of ferritin translation when iron is scarce. This precise mechanism allows for the rapid adjustment of iron-storage protein levels without the need for new transcription, ensuring an immediate cellular response.

**239.** (D) Cytoplasmic foci for mRNA decay and storage
Processing bodies, or P-bodies, are specialized cytoplasmic structures that play a key role in mRNA metabolism. They are sites where non-translating mRNAs accumulate and can be either stored for later use or targeted for degradation. P-bodies contain high concentrations of enzymes involved in deadenylation, decapping, and exonucleolytic decay, making them important hubs for the control of mRNA stability and overall gene expression.

**240.** (A) III, IV, I, II
The major pathway for mRNA decay follows a logical sequence of events. It starts with deadenylation, which leads to the loss of poly(A) binding proteins. This loss makes the transcript susceptible to decapping by the Dcp1/Dcp2 complex. Finally, once the 5' cap is removed, the RNA is rapidly degraded from the 5' end by the Xrn1 exonuclease, completing the destruction of the transcript.

**241.** (B) It acts as the primary binding site for TFIID to establish the transcription initiation site
The TATA box is a conserved DNA sequence found in the core promoter of many eukaryotic genes. It is recognized by the TATA-binding protein, a subunit of the general transcription factor TFIID. This binding event is essential for establishing the transcription initiation site, as it serves as a scaffold for the recruitment of other factors and RNA polymerase II to the promoter.

**242.** (D) Enhancer-promoter activation is blocked
Insulators are regulatory elements that establish boundaries between different genetic regions. When an insulator is positioned between an enhancer and a promoter, it physically or functionally blocks the enhancer's ability to activate that specific promoter. This mechanism ensures that enhancers only regulate their intended target genes and prevents inappropriate activation of neighboring genes, which is vital for the maintenance of precise gene expression.

**243.** (A) Relaxed chromatin and easier transcription
Histone acetyltransferases are enzymes that add acetyl groups to the lysine residues of histone tails. This modification neutralizes the positive charge on the histones, which weakens their electrostatic interaction with the negatively charged DNA. As a result, the chromatin structure becomes more relaxed and open, making the DNA more accessible to transcription factors and RNA polymerase, which directly facilitates the activation of transcription.

**244.** (C) Nonsense mutation
A nonsense mutation is a type of point mutation where a single nucleotide change converts a sense codon, which specifies an amino acid, into a stop codon. For example, changing a UAC (tyrosine) codon to UAA (stop) causes the ribosome to terminate translation prematurely. This results in the production of a truncated protein that is usually non-functional and often targeted for rapid degradation.

**245.** (A) The specific protein release factor
Translation termination is triggered when the ribosome encounters a stop codon in its A site. Since no tRNA matches this codon, a protein release factor binds instead. This factor alters the activity of the peptidyl transferase center, causing it to hydrolyze the ester bond that links the completed polypeptide to the tRNA in the P

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complex, the eIF4E protein is responsible for directly recognizing and binding to the 5' cap of the mRNA. This interaction serves as the anchor that recruits the other components of the complex and the small ribosomal subunit to the messenger RNA, marking the beginning of the initiation process.

**174.** (C) Internal Ribosome Entry Site (IRES)
While most eukaryotic mRNAs require a 5' cap for translation, some can utilize an alternative mechanism called an Internal Ribosome Entry Site. An IRES is a complex secondary structure within the mRNA that allows the ribosome to bind directly to an internal position, bypassing the need for a cap and the scanning process. This mechanism is frequently used by viruses to hijack cellular machinery.

**175.** (D) eIF2, GTP, and Met-tRNAiMet
The 43S pre-initiation complex is a key intermediate in eukaryotic translation initiation. A critical component of this complex is the ternary complex, which consists of the initiation factor eIF2 bound to a molecule of GTP and the initiator methionine-tRNA. This complex is recruited to the 40S subunit, where it prepares the ribosome to recognize the start codon once it binds to the mRNA.

**176.** (C) The 48S initiation complex is formed when the 60S subunit joins the 40S subunit
In eukaryotic translation, the 48S initiation complex is formed when the 43S pre-initiation complex successfully binds to the mRNA and locates the start codon through the scanning process. This complex does not yet include the large 60S subunit. The joining of the 60S subunit is a subsequent step that occurs only after the initiation factors are released and GTP is hydrolyzed.

**177.** (B) eIF4E
During certain viral infections, the cell's normal cap-dependent translation is inhibited to favor viral protein synthesis. This is often achieved by the viral-mediated cleavage or inactivation of eIF4E or the eIF4G scaffold. By bypassing the need for these factors, viruses can utilize their own Internal Ribosome Entry Sites to ensure that their mRNAs are translated even when the host's standard initiation machinery is compromised.

**178.** (D) eIF4G : serves as a scaffold interacting with eIF4E and PABP
eIF4G is a large scaffold protein that is essential for the assembly of the eukaryotic translation initiation complex. It contains multiple binding domains that allow it to interact with the cap-binding protein eIF4E, the RNA helicase eIF4A, and the poly(A) binding protein. By bridging these different components, eIF4G facilitates the circularization of the mRNA and coordinates the recruitment of the ribosome.

**179.** (A) GTP hydrolysis by eIF2 triggered by eIF5
Once the scanning 40S subunit identifies the correct AUG start codon, a critical regulatory step occurs. The initiation factor eIF5 acts as a GTPase-activating protein for eIF2, triggering the hydrolysis of its bound GTP. This event causes a conformational change that leads to the release of several initiation factors from the ribosome. This cleared state allows the large 60S subunit to join and begin elongation.

**180.** (A) 43S PIC formation → eIF4F assembly on mRNA → Scanning → 48S complex formation → 80S assembly
Eukaryotic translation initiation follows a precise sequence of events. It begins with the formation of the 43S pre-initiation complex. Simultaneously, the eIF4F complex assembles on the mRNA cap. The ribosome then binds and scans the mRNA until it finds the start codon, forming the 48S complex. Finally, the initiation factors are released, and the 60S subunit joins to form the functional 80S ribosome.

NODIA

**181.** (A) An aminoacyl-tRNA binds to the A site based on codon-anticodon complementarity
Decoding is the first stage of the translation elongation cycle. During this step, an aminoacyl-tRNA, delivered by an elongation factor, enters the ribosomal A site. The ribosome then monitors the complementarity between the tRNA's anticodon and the mRNA's codon. If a correct match is achieved, the tRNA is stably bound, preparing the ribosome for the subsequent step of peptide bond formation.

**182.** (D) EF-Tu
EF-Tu is a fundamental elongation factor in prokaryotes that ensures the accuracy and efficiency of protein synthesis. It binds to an aminoacyl-tRNA and a molecule of GTP, forming a ternary complex. This complex delivers the

# CHAPTER 06
# Molecular Biology Techniques

2023

**1.** What physical phenomenon causes the hyperchromic shift observed during nucleic acid denaturation?
(A) Disruption of hydrogen bonds and unstacking of nitrogenous bases
(B) Formation of covalent cross-links between complementary strands
(C) Hydrolysis of phosphodiester bonds by the application of heat
(D) Increased solvent polarity leading to protonation of the bases

2017

**2.** Which compound in the CTAB extraction buffer is primarily responsible for the denaturation of proteins and lysis of cell membranes?
(A) Ethylenediaminetetraacetic acid
(B) B-mercaptoethanol
(C) Cetyltrimethylammonium bromide
(D) Tris-HCl

2008

**3.** During alkaline lysis for plasmid isolation, what is the specific biochemical role of potassium acetate?
(A) It lowers the pH, causing genomic DNA and proteins to precipitate.
(B) It acts as a chelating agent to neutralize nucleases.
(C) It cleaves the circular plasmid DNA into linear fragments.
(D) It solubilizes the lipid bilayer of the bacterial cell wall.

2019

**4.** In the guanidinium thiocyanate-phenol-chloroform RNA extraction method, what ensures the partitioning of RNA into the aqueous phase rather than the organic phase?
(A) The high concentration of ethanol added
(B) The alkaline pH of the phenol mixture
(C) The presence of oligo-dT beads
(D) The acidic pH of the extraction medium

2003

**5.** Calculate the approximate concentration of a double-stranded DNA sample if its absorbance at 260 nm is 0.5 using a standard 1 cm path length cuvette.
(A) 20 µg/mL
(B) 25 µg/mL
(C) 33 µg/mL
(D) 50 µg/mL

2012

**6.** Which statement INCORRECTLY describes the function of loading dyes in agarose gel electrophoresis?
(A) They provide density to the sample to sink into the well.
(B) They allow visual tracking of the electrophoresis progress.
(C) They bind directly to DNA to induce fluorescence under UV light.
(D) They do not affect the intrinsic electrophoretic mobility of the DNA.

2010

**7.** In SDS-PAGE, what is the primary purpose of the stacking gel?
(A) To resolve proteins based on their differing molecular weights
(B) To provide a highly dense matrix that retards large complexes
(C) To neutralize the intrinsic negative charge of the sample proteins
(D) To concentrate protein samples into a narrow band before resolution

2007

**8.** Why is depurination often performed prior to the transfer step in Southern blotting?
(A) To fragment DNA, facilitating easier transfer to the membrane
(B) To permanently crosslink DNA using ultraviolet radiation
(C) To remove pyrimidine dimers and restore the DNA sequence
(D) To label the target DNA with specific radioactive isotopes

2006

**9.** In Northern blotting, what is the primary function of formaldehyde in the agarose gel?

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2008

**27.** What is the fundamental mechanism by which silica-based columns purify plasmid DNA in the final steps of alkaline lysis?
(A) Binding of DNA to silica in the presence of high concentrations of chaotropic salts
(B) Hydrophobic interactions under high salt conditions
(C) Electrostatic interactions under low pH conditions
(D) Covalent bonding between DNA phosphate groups and silica matrices

2016

**28.** Which of the following is a disadvantage of using the oligo-dT affinity chromatography method for mRNA purification?
(A) It fails to capture high-abundance ribosomal RNA.
(B) It isolates genomic DNA along with target mRNA.
(C) It requires intact poly-A tails, thus missing degraded mRNA.
(D) It degrades RNA during the competitive elution phase.

2017

**29.** Fluorometric quantification of DNA using dyes like PicoGreen is advantageous over UV spectrophotometry because it:
(A) Does not require the DNA sample to be in solution.
(B) Measures the exact sequence of the target molecules.
(C) Allows for the simultaneous quantification of proteins.
(D) Is highly specific to dsDNA and less affected by free nucleotides.

2022

**30.** Based on the following statements regarding agarose gel electrophoresis, select the correct option:
I. Higher agarose concentrations are used to resolve smaller DNA fragments.
II. TBE buffer provides better resolution for small DNA fragments than TAE.
III. Ethidium bromide intercalates between DNA base pairs and migrates towards the negative electrode.
Which of the above statements is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I only

2015

**31.** During SDS-PAGE, what dictates the uniform negative charge imparted to all proteins?
(A) The presence of intrinsic acidic amino acids in the sequence.
(B) Binding of SDS at a constant ratio of 1.4 g SDS per gram of protein.
(C) The alkaline pH of the resolving gel buffer during the run.
(D) The use of β-mercaptoethanol in the sample loading buffer.

2012

**32.** In Southern blotting, what is the specific role of the neutralization buffer used after denaturation?
(A) To re-anneal the double-stranded DNA before transfer
(B) To lower the pH to prevent the DNA from degrading during crosslinking
(C) To neutralize the negative charge on the DNA backbone
(D) To stabilize the single-stranded DNA for efficient capillary transfer

NODIA

2008

**33.** Which component must be excluded from a Northern blotting protocol that is otherwise standard for Southern blotting?
(A) Depurination with dilute HCl
(B) Gel electrophoresis
(C) Capillary transfer
(D) Membrane crosslinking

2010

**34.** In a Western blot, what is the most likely consequence of inadequately blocking the nitrocellulose membrane?
(A) The primary antibody will fail to bind its specific target protein.
(B) The secondary antibody will lose its enzymatic conjugate.
(C) High background signal will appear due to non-specific antibody binding.
(D) The transferred proteins will detach from the membrane during washing.

2019

**35.** Which of the following changes would most likely improve the specificity of a PCR reaction producing multiple off-target bands?
(A) Increasing the annealing temperature
(B) Increasing the extension time
(C) Decreasing the annealing temperature
(D) Increasing the primer concentration

2022

**18.** Which Next-Generation Sequencing (NGS) platform measures the release of protons ($H^+$) during nucleotide incorporation?
(A) Illumina sequencing-by-synthesis
(B) PacBio SMRT sequencing
(C) Ion Torrent sequencing
(D) Pyrosequencing

2016

**19.** In hybridoma technology, why do unfused myeloma cells die in the HAT selection medium?
(A) They lack the enzyme hypoxanthine-guanine phosphoribosyltransferase.
(B) They are susceptible to high concentrations of aminoglycoside antibiotics.
(C) They lack the biosynthetic machinery for purine regeneration.
(D) They possess an overactive metabolic inhibitor of thymidine kinase.

2013

**20.** Based on the characteristics of ELISA variants, match List I with List II:
List I: (ELISA variants)
P. Direct ELISA
Q. Indirect ELISA
R. Sandwich ELISA
S. Competitive ELISA
List II: (Distinguishing characteristics)
1. Antigen is trapped between two specific antibodies.
2. Labeled antibody directly binds to the immobilized antigen.
3. Highest signal indicates the lowest concentration of target antigen.
4. Unlabeled primary antibody binds antigen, then a labeled secondary antibody binds the primary.
(A) P-2, Q-4, R-1, S-3
(B) P-1, Q-3, R-2, S-4
(C) P-2, Q-1, R-4, S-3
(D) P-4, Q-2, R-3, S-1

2011

**21.** In a standard Radioimmunoassay (RIA), what is the relationship between the concentration of unlabeled patient antigen and the radioactivity of the bound fraction?
(A) Radioactivity increases linearly with patient antigen concentration.
(B) Radioactivity is inversely proportional to patient antigen concentration.
(C) Radioactivity remains constant regardless of patient antigen concentration.
(D) Radioactivity increases exponentially with patient antigen concentration.

2019

**22.** Which process is essential to allow the labeled FISH probe to access its target chromosomal DNA?
(A) Recombination
(B) Ligation
(C) Denaturation
(D) Transcription

2004

**23.** The Svedberg unit (S), used to describe ribosomal subunits in ultracentrifugation, is fundamentally a measure of what?
(A) Total molecular weight of the ribosomal complex
(B) Physical density of the particle relative to water
(C) Migration time required to reach the tube bottom
(D) Sedimentation velocity per unit of centrifugal force

2010

**24.** Which chromatographic matrix is most appropriate for isolating a protein based strictly on its specific biochemical interaction with a substrate?
(A) Nickel-NTA agarose
(B) DEAE-cellulose
(C) Sephadex G-100
(D) Carboxymethyl-cellulose

2004

**25.** Which ratio indicates significant protein contamination in a purified nucleic acid sample?
(A) $\frac{A_{260}}{A_{280}}$ ratio of 1.8
(B) $\frac{A_{260}}{A_{280}}$ ratio of 1.2
(C) $\frac{A_{260}}{A_{280}}$ ratio of 2.0
(D) $\frac{A_{260}}{A_{230}}$ ratio of 2.1

2012

**26.** Which chemical reagent in genomic DNA extraction causes the precipitation of proteins by unfolding them and exposing their hydrophobic regions?
(A) Isopropanol
(B) Phenol
(C) SDS
(D) RNase A

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2022

**46.** What is the primary advantage of using a fluorescently labeled bacterial artificial chromosome (BAC) probe over a small oligonucleotide probe in FISH?
(A) Higher resolution for detecting single nucleotide polymorphisms.
(B) Faster penetration of the nuclear membrane during hybridization.
(C) Stronger signal by covering a large contiguous chromosomal region.
(D) Requirement for target DNA denaturation is completely avoided.

2001

**47.** In density gradient ultracentrifugation using cesium chloride (CsCl), DNA molecules separate primarily according to their:
(A) Stokes radius
(B) Buoyant density
(C) Overall negative charge
(D) Sedimentation coefficient only

2023

**48.** Gel filtration chromatography separates molecules predominantly on the basis of:
(A) Isoelectric point
(B) Hydrophobicity
(C) Biospecific affinity
(D) Molecular size and shape

2018

**49.** In calculating the melting temperature ($T_m$) of a short oligonucleotide probe, which mathematical approximation is commonly used?
(A) $T_m = 4(G+C)+2(A+T)$
(B) $T_m = 2(G+C)+4(A+T)$
(C) $T_m = \dfrac{4(G+C)}{2(A+T)}$
(D) $T_m = \dfrac{A+T}{G+C}$

2005

**50.** Based on the following statements regarding RNA extraction, select the correct option:
I. Guanidinium thiocyanate acts as a potent RNase inhibitor.
II. Chloroform is added to separate the homogenate into distinct aqueous and organic phases.
III. RNA partitions into the organic phase under acidic conditions.
IV. Isopropanol is used to precipitate RNA from the aqueous phase.
Which of the above statements is/are correct?
(A) I, II and IV
(B) II, III and IV
(C) I and III
(D) I, II and III

2024

**51.** The primary structural difference between genomic DNA and plasmid DNA that allows for their differential separation during alkaline lysis is:
(A) Genomic DNA is linear while plasmid DNA is circular and supercoiled.
(B) Genomic DNA completely lacks histone-like organizing proteins.
(C) Plasmid DNA contains a significantly higher GC base pair content.
(D) Genomic DNA is localized exclusively within the bacterial nucleoid.

2002

**52.** Phenol used in nucleic acid extraction is typically equilibrated with Tris buffer to a pH of 8.0 because:
(A) Alkaline phenol degrades RNA through a base-catalyzed mechanism.
(B) Neutral pH effectively prevents the auto-oxidation of phenol.
(C) It matches the intracellular pH of typical bacterial host cells.
(D) Acidic phenol causes DNA to partition into the organic phase.

2018

**53.** The Beer-Lambert law equates absorbance ($A$) to the product of the molar extinction coefficient ($\varepsilon$), the path length ($l$), and the:
(A) Solvent density
(B) Solute concentration ($c$)
(C) Solution temperature
(D) Incident light intensity

2021

**54.** When comparing Native PAGE to SDS-PAGE, Native PAGE separates proteins based on:
(A) Molecular weight only
(B) Hydrophobicity and isoelectric point
(C) Charge-to-mass ratio and physical shape
(D) Subunit composition only

2019

**55.** What is the consequence of failing to use a neutralizing solution after soaking an agarose gel in NaOH during Southern blotting?
(A) The nylon membrane will fail to bind negatively charged DNA.
(B) The DNA will undergo spontaneous and fireball double-strand breaks.
(C) The DNA will immediately renature within

NODIA

2016

**36.** Which reverse transcriptase enzyme lacks inherent RNase H activity, making it superior for synthesizing long full-length cDNAs?
(A) AMV Reverse Transcriptase from avian sources.
(B) Taq DNA Polymerase used in standard PCR.
(C) T4 DNA Polymerase used for blunting ends.
(D) MMLV Reverse Transcriptase (RNase H minus mutant).

2022

**37.** In dye-based qPCR, what subsequent analysis is required to verify the specificity of the amplified product?
(A) Absolute standard curve generation
(B) Melt curve (dissociation) analysis
(C) Western blotting
(D) Southern blotting

2022

**38.** What parameter fundamentally limits the maximum insert size of a genomic fragment that can be packaged into a standard lambda phage vector?
(A) The host cell's restriction-modification system
(B) The efficiency of the DNA ligase enzyme
(C) The physical geometry of the phage head
(D) The origin of replication in the vector

2014

**39.** During the construction of a cDNA library, the second strand of DNA is typically synthesized using which combination of enzymes?
(A) Reverse transcriptase and RNase H
(B) Taq polymerase and Terminal transferase
(C) T4 DNA ligase and S1 nuclease
(D) RNase H, DNA Polymerase I, and DNA ligase

2007

**40.** Select the correct operational sequence for performing a colony hybridization screen:
(A) Immobilize cells on membrane → Lyse cells and denature DNA → Hybridize with probe → Autoradiography/Detection
(B) Lyse cells in liquid culture → Immobilize DNA on membrane → Hybridize with probe → Autoradiography/Detection
(C) Hybridize with probe → Immobilize cells on membrane → Lyse cells → Autoradiography/Detection
(D) Immobilize cells on membrane → Hybridize with probe → Lyse cells and denature DNA → Autoradiography/Detection

NODIA

2024

**41.** Capillary electrophoresis in modern Sanger sequencing separates DNA fragments based on:
(A) Charge-to-mass ratio using a pH gradient
(B) Size using a sieving polymer matrix
(C) Hydrophobicity using a non-polar solvent
(D) Fluorescence intensity

2003

**42.** Based on the following statements regarding Next-Generation Sequencing (NGS), select the correct option:
I. Pyrosequencing detects light emitted from a luciferase-catalyzed reaction.
II. Illumina sequencing uses reversible dye-terminator nucleotides.
III. PacBio SMRT sequencing requires the amplification of DNA on beads.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) I and III
(C) II and III
(D) III only

2021

**43.** The enzyme aminopterin in the HAT medium functions to:
(A) Block the de novo pathway of nucleotide synthesis.
(B) Promote the salvage pathway of nucleotide synthesis.
(C) Induce cell fusion between B-cells and myeloma cells.
(D) Selectively kill fused hybridoma cells.

2019

**44.** Which ELISA format is most appropriate for quantifying a small hapten molecule that cannot bind two antibodies simultaneously?
(A) Direct ELISA
(B) Indirect ELISA
(C) Competitive ELISA
(D) Sandwich ELISA

2019

**45.** In Radioimmunoassay (RIA), the sensitivity of the assay is most directly dependent on the:
(A) High concentration of the unlabeled antigen
(B) Use of a secondary antibody for signal amplification
(C) Volume of the sample utilized
(D) High specific activity of the radiolabeled antigen

(A) To increase the pore size of the gel matrix
(B) To prevent the formation of secondary structures in RNA
(C) To facilitate the binding of the RNA probe
(D) To stain the RNA for visualization under UV light

2005

**10.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): Polyclonal antibodies are rarely used as the primary antibody in precise therapeutic applications.
Reason (R): Polyclonal antibodies exhibit batch-to-batch variability and recognize multiple epitopes, increasing the risk of cross-reactivity.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**11.** Which factor is most likely to cause non-specific amplification during a Polymerase Chain Reaction (PCR)?
(A) Using an annealing temperature significantly below the primer $T_m$
(B) Using an excessively high annealing temperature for extension
(C) Significantly reducing the concentration of magnesium ions
(D) Decreasing the total number of thermal cycles used in the run

2023

**12.** In RT-PCR, which priming strategy is most appropriate for generating cDNA from eukaryotic mRNA while avoiding ribosomal RNA amplification?
(A) Random hexamer priming
(B) Sequence-specific priming
(C) Oligo-dT priming
(D) Poly-A priming

2014

**13.** How does the cycle threshold ($C_t$) value correlate with the initial template concentration in quantitative Real-Time PCR (qPCR)?
(A) It is directly proportional to the total initial template concentration.
(B) It remains completely independent of the initial template concentration.
(C) It increases exponentially as the initial template concentration rises.
(D) It is inversely proportional to the logarithm of the template concentration.

2005

**14.** Which restriction enzyme strategy is most suitable for generating a representative genomic library?
(A) Complete digestion using a rare-cutting restriction enzyme
(B) Partial digestion using a DNA ligase
(C) Partial digestion using a frequent-cutting restriction enzyme
(D) Complete digestion using an exonuclease

2013

**15.** During cDNA library construction, what is the function of adding linkers or adaptors to the double-stranded cDNA?
(A) To protect the cDNA from degradation by host nucleases
(B) To provide cohesive ends for insertion into a cloning vector
(C) To facilitate the transcription of the cDNA into mRNA
(D) To label the cDNA for subsequent screening

2013

**16.** Which of the following best differentiates plaque hybridization from colony hybridization?
(A) Plaque hybridization relies on immunological detection, while colony hybridization uses radioactive probes.
(B) Plaque hybridization requires specific antibodies, while colony hybridization requires RNA probes.
(C) Plaque hybridization is performed on liquid cultures, while colony hybridization is done on solid agar.
(D) Plaque hybridization screens bacteriophage libraries, while colony hybridization screens plasmid libraries.

2001

**17.** In Sanger dideoxy DNA sequencing, the termination of the elongating DNA strand occurs because the ddNTPs lack which specific chemical group?
(A) 3'-hydroxyl
(B) 5'-phosphate
(C) 2'-hydroxyl
(D) Nitrogenous base

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the agarose gel pores.
(D) High pH prevents the hybridization of the DNA probe later.

2001

**56.** Order the steps of a typical Western Blot procedure correctly:
I. Transfer to PVDF membrane
II. Blocking with non-fat milk
III. SDS-PAGE
IV. Incubation with secondary antibody
V. Incubation with primary antibody
(A) III, I, II, V, IV
(B) III, II, I, V, IV
(C) I, III, II, IV, V
(D) III, I, V, II, IV

2012

**57.** Which component of the PCR master mix provides the specific initiation site for Taq polymerase?
(A) Deoxynucleotide triphosphates (dNTPs)
(B) Oligonucleotide primers
(C) Magnesium chloride
(D) PCR buffer

2004

**58.** Absolute quantification in qPCR requires the construction of a:
(A) A melting curve to verify specific product amplification.
(B) Standard curve using known concentrations of template.
(C) A reference gene normalization profile using housekeepers.
(D) A logarithmic amplification plot of fluorescence signals.

2011

**59.** The incorporation of a "poly-A tail" at the 3' end of mRNA molecules allows for efficient first-strand cDNA synthesis using:
(A) Oligo(dT) primers
(B) Random hexamers
(C) Sequence-specific primers
(D) Terminal deoxynucleotidyl transferase

2008

**60.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): In vitro packaging is an essential step for constructing libraries using lambda phage vectors.
Reason (R): Naked lambda phage DNA cannot efficiently penetrate the bacterial cell wall via transformation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**61.** A cDNA library differs fundamentally from a genomic library in that a cDNA library:
(A) Contains introns and regulatory elements.
(B) Can only be constructed using prokaryotic cells.
(C) Always utilizes plasmid vectors rather than phages.
(D) Represents only the transcribed sequences of a specific tissue.

2007

**62.** Which labeled probe would be least relevant for screening a newly constructed cDNA library?
(A) A homologous DNA sequence from a related species
(B) A synthetic degenerate oligonucleotide based on a known protein sequence
(C) A randomly generated 6-base pair RNA fragment
(D) A specific antibody directed against the target protein

2004

**63.** During the analysis of a Sanger sequencing chromatogram, a position exhibiting two overlapping peaks of different colors most likely indicates:
(A) A sequencing artifact caused by excessive primer dimers.
(B) A heterozygous locus or a single nucleotide polymorphism.
(C) Premature termination by the polymerase during extension.
(D) Complete depletion of dNTPs in the sequencing reaction mix.

2023

**64.** Illumina sequencing-by-synthesis relies on the specific function of which proprietary molecule?
(A) Biotinylated dideoxynucleotides
(B) Apyrase enzymes
(C) Zero-mode waveguides
(D) Reversible dye-terminator nucleotides

NODIA

choose the most appropriate answer from the options provided:
Assertion (A): Partial restriction digestion is preferred over complete digestion when constructing a genomic library.
Reason (R): Partial digestion generates overlapping DNA fragments, ensuring that entire gene sequences are represented intact within the library.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**85.** During library construction, alkaline phosphatase treatment of the cloning vector is performed to:
(A) Facilitate the insertion of the cDNA fragment.
(B) Prevent self-ligation of the vector.
(C) Digest any contaminating genomic DNA.
(D) Enhance the transformation efficiency of the host cells.

2002

**86.** Immunological screening of an expression library relies on the synthesis of the target protein by the bacterial host, which requires the cloning vector to possess a:
(A) Eukaryotic origin of replication
(B) Telomeric sequence
(C) Strong prokaryotic promoter
(D) Centromere

2006

**87.** In an automated Sanger sequencing electropherogram, a broad, overlapping, multi-colored noise signal at the very beginning of the read is typically caused by:
(A) Strong secondary structures in the template DNA.
(B) DNA polymerase slippage during the reaction.
(C) Complete depletion of the DNA template molecules.
(D) Unincorporated dye terminators and short primers.

2002

**88.** Pyrosequencing detects nucleotide incorporation through an enzymatic cascade that ultimately converts luciferin to oxyluciferin, generating light. Which enzyme initiates this cascade by converting pyrophosphate (PPi) to ATP?
(A) ATP sulfurylase
(B) Luciferase
(C) Apyrase
(D) DNA polymerase

2006

**89.** Polyethylene glycol (PEG) is utilized in hybridoma technology primarily to:
(A) Selectively destroy unfused B-cells in the culture.
(B) Promote fusion of B-cell and myeloma plasma membranes.
(C) Provide a necessary carbon source for the hybrid cells.
(D) Effectively prevent bacterial contamination of the culture.

2016

**90.** Determine the correct operational sequence for performing a Sandwich ELISA:
(A) Add capture antibody → Block plate → Add sample antigen → Add detection antibody → Add substrate
(B) Add sample antigen → Block plate → Add capture antibody → Add detection antibody → Add substrate
(C) Add capture antibody → Add sample antigen → Block plate → Add detection antibody → Add substrate
(D) Add detection antibody → Block plate → Add sample antigen → Add capture antibody → Add substrate

2017

**91.** In RIA, the method utilized to separate the bound fraction from the free fraction must:
(A) Not disrupt the equilibrium between bound and free antigen.
(B) Completely denature the antibody-antigen complex.
(C) Utilize high heat to precipitate the sample proteins.
(D) Involve the addition of a highly concentrated salt solution.

2014

**92.** The DAPI stain used in conjunction with FISH serves to:
(A) Bind selectively to RNA to indicate active transcription sites.
(B) Crosslink the fluorescent DNA probe to the chromosome.
(C) Counterstain all DNA for context of the

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(D) Maintains the native secondary structure of mRNA species.

2023

**75.** Which assumption is required when using the A260 value to quantify RNA concentrations?
(A) Sample is free of DNA, as DNA also absorbs at 260 nm.
(B) The RNA is completely degraded into single nucleotide units.
(C) The sample contains a very high concentration of proteins.
(D) The RNA molecules in the sample are perfectly double-stranded.

2007

**76.** TAE buffer is generally preferred over TBE buffer for agarose gel electrophoresis when:
(A) High resolution of small DNA fragments is required.
(B) Electrophoresis must be run for an extended duration.
(C) The DNA fragments are to be extracted and recovered from the gel.
(D) The gel matrix concentration is above 2%.

2014

**77.** The tracking dye bromophenol blue typically migrates at a rate equivalent to a double-stranded DNA fragment of approximately:
(A) 50 base pairs in a 1% agarose gel
(B) 300 base pairs in a 1% agarose gel
(C) 4000 base pairs in a 1% agarose gel
(D) 10000 base pairs in a 1% agarose gel

2003

**78.** UV crosslinking fixes DNA to a nylon membrane by forming covalent bonds between the membrane and:
(A) The phosphate backbone
(B) Deoxyribose sugars
(C) Thymine residues
(D) Guanine residues

2004

**79.** Glyoxal is utilized in Northern blotting to:
(A) Hydrolyze the RNA molecules to ensure equal gel transfer.
(B) Form adducts with RNA bases to prevent secondary structures.
(C) Act as a fluorescent probe for direct visual RNA detection.
(D) Crosslink the RNA molecules to the nitrocellulose membrane.

2003

**80.** Based on the following statements regarding Western blotting, select the correct option:
I. PVDF membranes require activation in methanol before use.
II. Tween-20 is often added to wash buffers to reduce non-specific binding.
III. Chemiluminescent detection relies on the emission of light by a fluorophore excited by UV.
IV. BSA or non-fat milk can be used as blocking agents.
V. Nitrocellulose membranes have higher mechanical strength than PVDF membranes.
Which of the above statements is/are correct?
(A) I, II and IV
(B) II, III and V
(C) I, III, IV and V
(D) I, II and V

NODIA

2002

**81.** In PCR primer design, ensuring that the 3' end of the primer terminates in G or C (a GC clamp) is beneficial because:
(A) Promotes stronger hydrogen bonding at the polymerase site.
(B) Effectively prevents the formation of primer dimers.
(C) Significantly lowers the overall Tm of the primer.
(D) Prevents the polymerase from adding an extra adenine base.

2008

**82.** Which component is NOT required for a standard first-strand cDNA synthesis reaction?
(A) Reverse transcriptase
(B) dNTPs
(C) DNA ligase
(D) An oligonucleotide primer

2006

**83.** The major disadvantage of using SYBR Green I in qPCR compared to TaqMan probes is that SYBR Green I:
(A) Binds to any double-stranded DNA, including primer dimers.
(B) Cannot quantify DNA in real-time.
(C) Requires complex optimization of magnesium concentrations.
(D) Is rapidly degraded by Taq polymerase during extension.

2004

**84.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these,

2001

**65.** The primary purpose of performing a serial dilution of hybridoma cells following HAT selection is to:
(A) Isolate single-cell clones to ensure true monoclonality.
(B) Ensure the cells receive adequate nutrients.
(C) Eliminate residual myeloma cells.
(D) Induce the secretion of the monoclonal antibodies.

2011

**66.** In an Indirect ELISA, the enzyme is conjugated to the:
(A) Solid-phase well
(B) Target antigen
(C) Secondary antibody
(D) Primary antibody

2005

**67.** The fundamental principle of a Radioimmunoassay (RIA) is the competitive binding between:
(A) Radiolabeled and unlabeled antigen for a specific antibody.
(B) Two different primary antibodies for the same target antigen.
(C) Two different antigens for a single radiolabeled antibody.
(D) A primary antibody and a radiolabeled secondary antibody species.

2016

**68.** Which parameter must be strictly controlled during the hybridization step of FISH to prevent non-specific probe binding?
(A) Photobleaching
(B) The size of the fluorescent molecule
(C) The concentration of the DAPI counterstain
(D) Stringency (temperature and formamide concentration)

2001

**69.** Differential centrifugation fractionates cellular organelles primarily on the basis of their:
(A) Electric charge
(B) Specific gravity and mass
(C) Hydrophobic interactions
(D) Binding affinity to the tube walls

2002

**70.** Based on the following statements regarding chromatography matrices, select the correct option:
I. Cation-exchange matrices bind positively charged proteins.
II. Sephadex is commonly used in affinity chromatography.
III. Anion-exchange matrices possess a positive surface charge.
IV. Gel filtration matrices exclude large molecules, causing them to elute first.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II and III
(C) II only
(D) I, III and IV

2017

**71.** The $\frac{A_{260}}{230}$ ratio of a DNA sample is evaluated to detect contamination by:
(A) Proteins and aromatic amino acids
(B) Ribosomal RNA
(C) Free nucleotides
(D) Chaotropic salts, phenol, and carbohydrates

2016

**72.** In the extraction of genomic DNA, absolute ethanol is added to the aqueous phase primarily to:
(A) Decreases the dielectric constant, precipitating DNA.
(B) Denatures remaining proteins by unfolding them completely.
(C) Solubilizes lipid contaminants in the cell membrane.
(D) Neutralizes the pH of the initial genomic extraction buffer.

2003

**73.** During plasmid isolation, violent vortexing after the addition of the alkaline lysis buffer should be avoided because it can:
(A) Causes premature precipitation of the cellular proteins.
(B) Shears genomic DNA, leading to plasmid contamination.
(C) Denatures the plasmid DNA molecules irreversibly.
(D) Completely inactivates the added RNase A enzyme.

2005

**74.** Which role does β-mercaptoethanol play when included in RNA extraction buffers?
(A) Precipitates the RNA by reducing total solubility.
(B) Breaks disulfide bonds, irreversibly denaturing RNases.
(C) Enhances the binding of RNA to the silica-based column.

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probe signal.
(D) Effectively quench background fluorescence in the sample.

2023

**93.** Analytical ultracentrifugation is best distinguished from preparative ultracentrifugation by its ability to:
(A) Isolate large quantities of pure ribosomal subunits.
(B) Utilize continuous sucrose density gradients for runs.
(C) Reach significantly higher maximum rotational speeds.
(D) Monitor sedimentation in real-time using optical systems.

2025

**94.** In ion-exchange chromatography, proteins bound to the matrix are typically eluted by:
(A) Passing a highly hydrophobic solvent through the column.
(B) Adding a specific competitive substrate for the protein.
(C) Gradually increasing the salt concentration of the phase.
(D) Decreasing the column temperature during the elution step.

2010

**95.** The hyperchromic effect is quantitatively evaluated by monitoring absorbance at 260 nm. What is the typical increase in $A_{\{260\}}$ when double-stranded DNA is completely denatured into single strands?
(A) Approximately 5-10%
(B) Approximately 30-40%
(C) Approximately 80-90%
(D) Approximately 100-120%

2006

**96.** During DNA extraction using phenol-chloroform-isoamyl alcohol (25:24:1), what is the primary function of isoamyl alcohol?
(A) Acts as a co-solvent to dissolve the target DNA.
(B) Rapidly degrades any residual cellular RNA.
(C) Effectively inhibits all endogenous nucleases.
(D) Prevents foaming and aids in sharp phase separation.

2005

**97.** In the context of alkaline lysis for plasmid extraction, prolonged exposure to NaOH should be avoided because:
(A) It may cause irreversible denaturation of the plasmid DNA.
(B) It will cause the SDS to precipitate prematurely.
(C) It will neutralize the added potassium acetate buffer.
(D) It will rapidly lyse the cellular mitochondria.

2003

**98.** Based on the components of RNA extraction, match List I with List II:
List I: (Extraction components)
P. Guanidinium thiocyanate
Q. DEPC (Diethyl pyrocarbonate)
R. Oligo-dT cellulose
S. Isopropanol
List II: (Primary functions)
1. Precipitation of total RNA
2. Strong chaotropic agent and RNase denaturant
3. Chemical inactivation of RNases in water/ buffers
4. Specific isolation of polyadenylated mRNA
(A) P-2, Q-3, R-4, S-1
(B) P-3, Q-2, R-1, S-4
(C) P-2, Q-1, R-4, S-3
(D) P-4, Q-3, R-2, S-1

2016

**99.** Which of the following equations accurately calculates the purity of a DNA sample utilizing spectrophotometry?
(A) Purity $= \frac{A_{260}}{A_{280}}$
(B) Purity $= A_{260} \times A_{280}$
(C) Purity $= \frac{A_{280}}{A_{260}}$
(D) Purity $= \frac{A_{260} - A_{280}}{A_{230}}$

2003

**100.** Based on the following statements regarding DNA electrophoresis, select the correct option:
I. Gel loading dye contains glycerol or sucrose to increase sample density.
II. DNA migrates towards the cathode during agarose gel electrophoresis.
III. Ethidium bromide visualization requires a transilluminator.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II only
(C) III only
(D) I and III

NODIA

(C) RNA is highly susceptible to ubiquitous and stable RNases.
(D) RNA lacks the negative charge necessary for electrophoresis.

2006

**175.** The transfer of proteins from a polyacrylamide gel to a nitrocellulose membrane in Western blotting is typically driven by:
(A) Capillary action using salt buffers
(B) An electrophoretic current (electroblotting)
(C) Vacuum pressure
(D) Gravitational flow

2023

**176.** Which component is specifically adjusted during PCR optimization to manipulate primer annealing stringency?
(A) The concentration of the deoxynucleotides.
(B) The extension time of the DNA polymerase.
(C) The total concentration of the DNA template used.
(D) Annealing temperature and magnesium ion concentration.

2025

**177.** In a one-step RT-PCR reaction, which statement is true?
(A) Process occurs in a single tube without opening it.
(B) The reverse transcriptase and polymerase are identical.
(C) It is more prone to contamination than the two-step.
(D) It allows for the generation of a stable cDNA library.

2002

**178.** TaqMan probes utilized in qPCR rely on which physical phenomenon to generate a fluorescent signal?
(A) Hyperchromic shift
(B) Intercalation
(C) Fluorescence Resonance Energy Transfer (FRET)
(D) Chemiluminescence

2013

**179.** A partial restriction digest of genomic DNA is typically achieved by manipulating:
(A) Enzyme concentration and incubation time
(B) The type of restriction enzyme used (blunt vs. sticky end)
(C) The sequence of the adapters added
(D) The type of ligase used

2017

**180.** Based on the following statements concerning cDNA library construction, select the correct option:
I. First-strand synthesis is catalyzed by reverse transcriptase.
II. Genomic DNA is used as the initial template.
III. Terminal transferase can be used to add homopolymeric tails.
Which of the above statements is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) III only

2023

**181.** Immunological screening identifies a positive clone based on the interaction between:
(A) A specific DNA probe and the plasmid DNA
(B) A specific antibody and the protein expressed by the clone
(C) The bacteriophage and the bacterial host receptors
(D) Two complementary DNA strands

2001

**182.** Based on the following statements regarding DNA sequencing, select the correct option:
I. Sanger sequencing utilizes DNA polymerase to extend a primer.
II. Dideoxynucleotides (ddNTPs) possess a 3'-OH group.
III. In capillary electrophoresis, shorter fragments pass the detector first.
IV. Chromatogram peaks of the same color represent the same nucleotide base.
Which of the above statements is/are INCORRECT?
(A) II only
(B) I and II
(C) II and III
(D) III and IV

2022

**183.** Which feature distinguishes PacBio SMRT sequencing from Illumina sequencing?
(A) It requires the generation of a clonal cluster via bridge amplification.
(B) It reads sequences by detecting changes in pH.
(C) It exclusively utilizes solid-phase reversible dye-terminators.
(D) It performs sequencing on single unamplified DNA molecules.

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(D) A is false but R is true.

2012

**111.** The distinct advantage of capillary electrophoresis over traditional slab gel electrophoresis in Sanger sequencing is:
(A) It eliminates the need for any fluorescent dyes.
(B) Faster separation and automated data collection.
(C) It requires large amounts of radioactive isotopes.
(D) It enables sequencing of entire genomes in one run.

2004

**112.** Based on the following statements regarding Single-Molecule Real-Time (SMRT) sequencing (PacBio), select the correct option:
I. It requires the amplification of DNA templates before sequencing.
II. It utilizes zero-mode waveguides (ZMWs) to detect fluorescence.
III. It can generate significantly longer read lengths compared to Illumina sequencing.
IV. Nucleotides are added one at a time and washed away between cycles.
V. It detects the incorporation of labeled nucleotides in real-time.
Which of the above statements is/are correct?
(A) II, III and V
(B) I, II, III and V
(C) II, III and IV
(D) I, IV and V

2004

**113.** In hybridoma production, what characterizes the myeloma cells chosen as fusion partners?
(A) Immortal, HGPRT-deficient, and do not secrete endogenous antibodies.
(B) They secrete large amounts of random non-specific antibodies.
(C) They are primary cells derived directly from the mouse spleen.
(D) They are hyper-sensitive to aminopterin but survive using salvage.

2001

**114.** A Competitive ELISA typically results in an assay response where high concentrations of the sample antigen yield a:
(A) High absorbance signal
(B) Color change from yellow to blue
(C) Low absorbance signal
(D) High fluorescence signal only

2025

**115.** Radiolabeling of proteins for use in RIA is most commonly achieved by incorporating Iodine-125 ($_{\{125\}}$I) into which amino acid residue?
(A) Tyrosine
(B) Alanine
(C) Glycine
(D) Serine

2004

**116.** Which technique provides the highest physical resolution for mapping a gene to a specific chromosomal band?
(A) Southern blotting
(B) Preparative ultracentrifugation
(C) Affinity chromatography
(D) Fluorescence In Situ Hybridization (FISH)

2021

**117.** In density gradient centrifugation, what happens to a particle when it reaches the zone where the gradient density matches its own buoyant density?
(A) It precipitates to the bottom of the tube.
(B) It stops migrating and forms a discrete band (isopycnic point).
(C) It reverses its direction of migration.
(D) It dissolves into the gradient medium.

2003

**118.** Which molecule would elute first from a gel filtration chromatography column?
(A) A 10 kDa globular protein
(B) A 50 kDa globular protein
(C) A 100 kDa globular protein
(D) A small unbound salt ion

2017

**119.** A DNA fragment with a high GC content will exhibit a higher melting temperature (Tm) than an AT-rich fragment of the same length because:
(A) G-C pairs form covalent bonds under physiological conditions.
(B) G-C pairs induce significant supercoiling of the DNA helix.
(C) G-C pairs are physically larger than A-T base pairs.
(D) Bonded by three hydrogen bonds, requiring more energy.

2012

**120.** Based on the principles of electrophoresis, match List I with List II:
List I: (Components)
P. Ethidium bromide
Q. SDS

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HGPRT. Why do unfused B-cells eventually die in culture despite this?
(A) The aminopterin physically damages their cell membranes.
(B) Intrinsically mortal and cannot survive long-term in vitro.
(C) They are killed by the adjacent myeloma cells in culture.
(D) The HAT medium lacks the necessary substrate hypoxanthine.

2002

**139.** In a Direct ELISA, the principal cause of high background noise is typically:
(A) Non-specific binding of labeled primary antibody to plate.
(B) Cross-reactivity of the secondary antibody with the sample.
(C) Competitive binding by unlabelled antigens in the mixture.
(D) Hydrolysis of the substrate by the blocking buffer proteins.

2006

**140.** Based on the following statements regarding FISH, select the correct option:
I. FISH can detect microdeletions that are invisible on a standard G-banded karyotype.
II. FISH probes are typically labeled with radioactive isotopes like ${}_{\{32\}}$P.
III. Interphase FISH does not require the cells to be in active division.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II only
(C) I and III
(D) II and III

2011

**141.** A protein's sedimentation coefficient (S) is directly proportional to its mass and inversely proportional to its:
(A) Density
(B) Isoelectric point
(C) Net charge
(D) Frictional coefficient (shape)

2018

**142.** Anion-exchange chromatography utilizes a matrix that is:
(A) Negatively charged to bind positively charged molecules
(B) Uncharged and highly hydrophobic
(C) Positively charged to bind negatively charged molecules
(D) Covalently bound to a specific substrate analog

2003

**143.** Which factor primarily dictates the calculated melting temperature ($T_m$) of a DNA duplex?
(A) The concentration of DNA in the solution
(B) The sequence length and its GC content
(C) The age of the DNA sample
(D) The pH of the extraction buffer used previously

NODIA

2025

**144.** In a phenol-chloroform extraction, if the pH of the phenol is roughly 4.5, what will be the distribution of nucleic acids?
(A) DNA and RNA will both partition into the aqueous phase.
(B) DNA will partition into the aqueous phase, while RNA partitions into the organic phase.
(C) Both DNA and RNA will partition into the organic phase.
(D) RNA will partition into the aqueous phase, while DNA partitions into the organic/interphase.

2013

**145.** What is the fundamental mechanism by which SDS lyses cells during plasmid extraction?
(A) It solubilizes the lipid bilayer of the cell membrane.
(B) It digests the cell wall peptidoglycan.
(C) It cleaves the genomic DNA into small fragments.
(D) It competitively inhibits ATP synthesis.

2025

**146.** Which of the following is a critical advantage of using guanidinium thiocyanate for RNA extraction?
(A) It stabilizes the tertiary structure of the RNA molecules.
(B) It selectively binds mRNA, leaving rRNA in the supernatant.
(C) Powerful chaotropic agent that denatures endogenous RNases.
(D) It functions as a reverse transcriptase during the run.

2013

**147.** Under standard assay conditions, an A260 reading of 1.0 corresponds to an RNA concentration of approximately:
(A) 20 µg/mL
(B) 33 µg/mL

(D) The molecular weight of its translated protein

2006

**130.** Based on the following statements concerning Reverse Transcription PCR (RT-PCR), select the correct option:

I. MMLV reverse transcriptase operates optimally at higher temperatures than AMV reverse transcriptase.

II. Random hexamers prime cDNA synthesis across the entire length of all RNA species.

III. Oligo-dT primers bind to the 5' cap of mRNA molecules.

Which of the above statements is/are correct?

(A) I and II
(B) II only
(C) I and III
(D) II and III

2014

**131.** In qPCR, the term "baseline" refers to:

(A) The background fluorescence level during the initial cycles of PCR.
(B) The threshold set for determining the $C_t$ value.
(C) The final plateau phase of amplification.
(D) The theoretical 100% efficiency of the polymerase.

2015

**132.** The primary reason for utilizing a DNA polymerase lacking 3' to 5' exonuclease proofreading activity is:

(A) Complete failure of the PCR amplification
(B) Inability to synthesize fragments longer than 100 bp
(C) Rapid degradation of the primer molecules
(D) A higher rate of nucleotide misincorporation

2002

**133.** Which parameter is critical for accurately calculating the titer of a newly constructed library?

(A) The volume of the extraction buffer used
(B) The number of plaques or colonies per volume of plated dilution
(C) The total concentration of ethidium bromide in the gel
(D) The ratio of RNA to DNA in the initial sample

2005

**134.** Linkers added during cDNA cloning often contain a specific restriction site. Why must the cDNA be protected (e.g., by methylation) before digesting these linkers?

(A) To prevent the restriction enzyme from degrading the vector.
(B) To ensure the linkers anneal properly to the cloning vector.
(C) Prevent enzyme from cutting internal sites in cDNA.
(D) To activate the ligase enzyme during the final step.

2024

**135.** Immunological screening of a cDNA expression library requires a primary antibody. How is the bound primary antibody subsequently visualized?

(A) By directly observing the plate under a UV transilluminator.
(B) By hybridizing a radioactive DNA probe to the bound complex.
(C) By performing a PCR amplification of the bound antibody.
(D) Applying secondary antibody followed by substrate.

2009

**136.** The chain-termination chemistry in Sanger sequencing is driven by dideoxynucleotides. Why does a polymerase stop extending when a ddNTP is incorporated?

(A) Lack of 3'-OH prevents phosphodiester bond formation.
(B) The ddNTP molecule sterically hinders the polymerase enzyme.
(C) The ddNTP is recognized as a termination signal by the enzyme.
(D) The ddNTP lacks a nitrogenous base to pair with the template.

2016

**137.** Which statement accurately compares Pyrosequencing to Sanger sequencing?

(A) Pyrosequencing requires capillary electrophoresis during the run.
(B) Detects light emission vs. size separation.
(C) Pyrosequencing is optimal for reading fragments over 1000 bp.
(D) Pyrosequencing strictly requires the use of chain-terminating ddNTPs.

2005

**138.** Aminopterin in the HAT medium blocks the de novo nucleotide synthesis pathway. Normal B-cells possess the salvage pathway enzyme

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long-term storage.

2018

**157.** Which screening method utilizes a labeled complementary sequence to identify a specific clone?
(A) Immunological screening
(B) Functional complementation
(C) Blue-white screening
(D) Plaque hybridization

2024

**158.** In Sanger sequencing, sequence reads are typically limited to ~800-1000 base pairs because:
(A) The polymerase loses processivity and dissociates from the DNA.
(B) The ddNTPs become entirely depleted after 1000 base cycles.
(C) Capillary electrophoresis fails to resolve single-base diff.
(D) The template DNA fragments spontaneously circularize in the mix.

2022

**159.** The Illumina sequencing platform employs "bridge amplification" to:
(A) Synthesize the complementary strand of a single template.
(B) Generate dense localized clusters of identical molecules.
(C) Connect two different sequencing reads together in sequence.
(D) Repair damaged DNA templates prior to the sequencing run.

2018

**160.** Based on the following statements concerning monoclonal antibodies (mAbs), select the correct option:
I. They are secreted by a population of identical B-cell hybridomas.
II. They bind to a single specific epitope on an antigen.
III. They are typically produced by injecting an animal and simply harvesting its serum.
IV. Hybridoma selection requires HAT medium to isolate successful fusions.
V. They demonstrate significant batch-to-batch variability.
Which of the above statements is/are correct?
(A) I, II and IV
(B) II, III, IV and V
(C) I, III and V
(D) I, II, III and IV

2023

**161.** A major advantage of an Indirect ELISA over a Direct ELISA is:
(A) Multiple secondary antibodies bind a single primary antibody.
(B) It requires fewer washing steps to complete the assay.
(C) It involves only one antibody, significantly reducing costs.
(D) It does not require an enzyme substrate for signal detection.

2019

**162.** In an RIA for evaluating thyroid hormone levels, a patient sample with an abnormally high hormone concentration will result in a:
(A) High amount of radioactivity in the bound complex
(B) Precipitation of the antibody
(C) Low amount of radioactivity in the bound complex
(D) Colorless solution

NODIA

2014

**163.** The primary role of formamide in a FISH hybridization buffer is to:
(A) Lower $T_m$, allowing hybridization at lower temperatures.
(B) Increase the melting temperature of the target DNA duplex.
(C) Act as a fluorescent tag for the labeled DNA probe.
(D) Digest RNA to effectively prevent background noise signals.

2019

**164.** What defines the "void volume" in gel filtration chromatography?
(A) The total volume of the liquid inside the porous beads.
(B) The volume of the mobile phase required to elute the smallest molecules.
(C) The total geometric volume of the empty column before packing.
(D) The volume of the mobile phase located outside the porous beads.

2018

**165.** During affinity chromatography of a His-tagged protein, imidazole is used in the elution buffer because:
(A) It lowers the pH, denaturing the target protein.
(B) Competitively displaces histidine from nickel on the matrix.
(C) It specifically cleaves the His-tag from the

(C) 40 μg/mL
(D) 50 μg/mL

2003

**148.** Agarose forms a gel matrix through which physical mechanism?
(A) Covalent crosslinking of polymer chains in the matrix.
(B) Ionic interactions between sulfate groups on the chain.
(C) Polymerization induced by free radicals in the solution.
(D) Hydrogen bonding and entanglements of polysaccharide chains.

2023

**149.** Native PAGE preserves the biological activity of proteins primarily because it omits:
(A) Acrylamide
(B) SDS and reducing agents
(C) APS (Ammonium persulfate)
(D) A continuous buffer system

2021

**150.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): Capillary transfer of DNA in Southern blotting is driven by the flow of transfer buffer from a reservoir through the gel and membrane to a stack of dry paper towels.
Reason (R): The DNA fragments are actively propelled toward the positively charged paper towels by an applied electrical field.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**151.** Which detection method is least suitable for a standard Northern blot?
(A) Radioactively labeled cDNA probe
(B) Digoxigenin (DIG) labeled cRNA probe
(C) Biotin-labeled oligonucleotide probe
(D) Fluorescently labeled specific antibody

2017

**152.** In Western blotting, milk proteins in the blocking solution prevent background signal by:
(A) Binding to all empty, unoccupied hydrophobic sites on membrane.
(B) Digesting unbound primary antibodies in the wash buffer.
(C) Providing a substrate for the horseradish peroxidase enzyme.
(D) Crosslinking target proteins to the PVDF membrane permanently.

2007

**153.** A PCR profile displays poor amplification of a GC-rich target. Which additive is commonly supplemented to the master mix to alleviate this problem?
(A) EDTA
(B) DMSO (Dimethyl sulfoxide)
(C) SDS
(D) Proteinase K

2016

**154.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): Real-time PCR (qPCR) is superior to end-point PCR for accurate quantification of target sequences.
Reason (R): qPCR measures amplification during the exponential phase, where reaction efficiency is consistent and components are not yet limiting.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**155.** Which library requires an expression vector containing a promoter and a Ribosome Binding Site (RBS)?
(A) A cDNA library intended for immunological screening
(B) A standard genomic DNA library screened by hybridization
(C) A BAC library for physical mapping
(D) A YAC library for sequencing

2018

**156.** In the context of library construction, "in vitro packaging" refers to:
(A) The synthesis of cDNA inside a test tube.
(B) The encapsulation of plasmids into liposomes for transformation.
(C) The assembly of recombinant lambda DNA into mature infectious phage particles.
(D) The process of freeze-drying the library for

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R. Acrylamide
S. Tracking dye
List II: (Molecular interactions)
1. Forms a polyanionic complex with proteins
2. Intercalates between planar base pairs
3. Co-migrates with the solvent front
4. Polymerizes to form a porous matrix
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-2, Q-4, R-1, S-3
(D) P-3, Q-1, R-4, S-2

2024

**121.** What is the fundamental function of the silica membrane inside a spin column used for genomic DNA purification?
(A) It chemically digests cellular RNA and protein content.
(B) Binds DNA reversibly in high chaotropic salt concentrations.
(C) It separates DNA fragments based strictly on molecular size.
(D) It neutralizes the pH of the final DNA elution buffer.

2011

**122.** During plasmid extraction, if the cellular lysate turns cloudy immediately after the addition of the neutralization buffer (potassium acetate), it indicates:
(A) The protocol has failed and the DNA is degraded.
(B) The successful precipitation of SDS, proteins, and genomic DNA.
(C) The contamination of the sample by RNA.
(D) The improper mixing of the lysis buffer.

2002

**123.** The function of chloroform in RNA extraction is primarily to:
(A) Extracts lipids and stabilizes phase separation.
(B) Precipitates the RNA from the aqueous solution phase.
(C) Denatures the secondary structure of RNA molecules.
(D) Chemically inactivates DEPC used in the buffer.

2019

**124.** When measuring RNA purity, a significantly low $\frac{A_{260}}{A_{280}}$ ratio (e.g., 1.4) strongly suggests contamination by:
(A) Carbohydrates
(B) Genomic DNA
(C) Proteins
(D) Phenol

2008

**125.** Which fluorophore is most specific for quantifying single-stranded DNA?
(A) PicoGreen
(B) RiboGreen
(C) Hoechst 33258
(D) OliGreen

2001

**126.** Order the sequence of steps for performing a Southern Blot correctly:
(A) Restriction digestion → Gel electrophoresis → Denaturation → Membrane transfer → Hybridization
(B) Gel electrophoresis → Restriction digestion → Membrane transfer → Denaturation → Hybridization
(C) Denaturation → Restriction digestion → Gel electrophoresis → Membrane transfer → Hybridization
(D) Restriction digestion → Gel electrophoresis → Membrane transfer → Hybridization → Denaturation

2008

**127.** Why are stacking gels cast with a lower percentage of acrylamide compared to resolving gels in SDS-PAGE?
(A) To separate the proteins tightly before they enter the resolving gel.
(B) Allow proteins to migrate rapidly and concentrate at the interface.
(C) To increase the mechanical strength of the well dividers used.
(D) To ensure the SDS binds uniformly to all the sample proteins.

NODIA

2023

**128.** In Southern blotting, a probe designed to detect a specific gene must be:
(A) Double-stranded during the hybridization step
(B) Covalently linked to the nylon membrane
(C) Identical to the target sequence
(D) Single-stranded and complementary to the target sequence

2016

**129.** Northern blotting is commonly employed to ascertain which property of a specific gene?
(A) Its expression level and transcript size
(B) Its precise chromosomal location
(C) Its primary nucleotide sequence

protein.
(D) It increases the hydrophobic interactions within the matrix.

**2009**

**166.** A DNA sample exhibits an $\frac{A_{260}}{A_{280}}$ ratio of 2.1. What is the most appropriate interpretation?
(A) The DNA sample is highly pure and ready for use.
(B) The DNA is heavily contaminated with protein impurities.
(C) Likely contains significant RNA contamination.
(D) The sample contains phenol carryover from the extraction.

**2015**

**167.** Which reagent is crucial for the efficient precipitation of DNA using ethanol?
(A) Ethylenediaminetetraacetic acid (EDTA) for chelation.
(B) Sodium dodecyl sulfate (SDS) for protein denaturation.
(C) Tris base for maintaining a stable alkaline pH.
(D) A monovalent cation (e.g., Sodium or Ammonium).

**2002**

**168.** Based on the reagents used for plasmid isolation, match List I with List II:
List I: (Lysis reagents)
P. Solution I (Glucose/Tris/EDTA)
Q. Solution II (NaOH/SDS)
R. Solution III (Potassium Acetate)
S. Isopropanol
List II: (Biochemical effects)
1. Neutralizes pH and precipitates genomic DNA
2. Concentrates the purified plasmid DNA
3. Osmotic stabilization and divalent cation chelation
4. Lysis of cell membrane and denaturation of DNA
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-1, Q-2, R-4, S-3
(D) P-3, Q-1, R-4, S-2

**2023**

**169.** Oligo-dT chromatography separates mRNA from rRNA based on:
(A) The significantly larger molecular weight of mRNA.
(B) Poly-A tail exclusively on eukaryotic mRNA.
(C) The specific 5' cap structure of the mRNA molecules.
(D) The significantly higher GC base pair content of rRNA.

**2024**

**170.** Which equation best defines the relationship between absorbance and transmission in spectrophotometry?
(A) $A = \frac{1}{T}$
(B) $A = \log\left(\frac{1}{T}\right)$
(C) $A = e^{T}$
(D) $A = 1 - T$

**2019**

**171.** Why is ethidium bromide considered a potent mutagen?
(A) Intercalates base pairs, causing frameshift mutations.
(B) It acts as a nuclease, cutting the DNA backbone.
(C) It covalently methylates specific cytosine residues.
(D) It strips histones from the genomic DNA complexes.

**2010**

**172.** During SDS-PAGE, β-mercaptoethanol or DTT is added to the sample loading buffer to:
(A) Denature the proteins by breaking peptide bonds.
(B) Coat the proteins with a uniform negative charge.
(C) Reduce intra- and inter-molecular disulfide bonds.
(D) Increase the density of the sample.

**2001**

**173.** What is the role of the depurination step (0.25 N HCl) prior to Southern blot transfer?
(A) It crosslinks the DNA to the gel matrix permanently.
(B) It neutralizes the agarose gel before the transfer.
(C) It denatures double-stranded DNA into single strands.
(D) Breaks DNA into smaller fragments to facilitate transfer.

**2007**

**174.** Northern blotting is more challenging than Southern blotting primarily because:
(A) RNA is smaller and difficult to trap on membranes.
(B) RNA is single-stranded and cannot hybridize.

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2001

**101.** Which resolving gel concentration is most suitable for separating small proteins (e.g., 10-15 kDa) in SDS-PAGE?
(A) 5%
(B) 8%
(C) 10%
(D) 15%

2007

**102.** Nitrocellulose membranes are often preferred over nylon membranes in specific blotting applications because they:
(A) Exhibit significantly higher physical strength and durability.
(B) Bind DNA covalently without the need for UV crosslinking.
(C) Lower background noise due to lower non-specific binding.
(D) Have a higher binding capacity for short oligonucleotides.

2025

**103.** The primary reason for utilizing formaldehyde in Northern blot electrophoresis is to ensure that the RNA molecules separate strictly based on:
(A) Base composition
(B) Secondary structure complexity
(C) Negative charge density
(D) Molecular weight

2008

**104.** In Western blotting, the secondary antibody is conjugated to an enzyme. Which enzyme is most widely used for chemiluminescent detection in this assay?
(A) Horseradish peroxidase (HRP)
(B) Taq polymerase
(C) B-galactosidase
(D) Alkaline phosphatase

2001

**105.** During PCR, the extension phase is typically performed at 72°C because:
(A) The primers anneal most stably at this specific temperature.
(B) Optimal temperature for the catalytic activity of Taq.
(C) The DNA template remains fully single-stranded at this point.
(D) It effectively prevents the degradation of the dNTP molecules.

2018

**106.** In a two-step RT-PCR protocol, which reaction is performed first?
(A) Amplification of cDNA using specific primers
(B) Reverse transcription of RNA into cDNA
(C) Digestion of genomic DNA
(D) Denaturation of the Taq polymerase

NODIA

2017

**107.** Which mathematical method is widely used for the relative quantification of target gene expression in qPCR, utilizing a reference gene?
(A) The $2^{-\Delta\Delta C_t}$ method
(B) The Beer-Lambert formula
(C) The Svedberg equation
(D) The Henderson-Hasselbalch equation

2021

**108.** A genomic library is conceptually distinct from a chromosome-specific library in that a genomic library:
(A) Contains DNA from only one sorted chromosome.
(B) Only contains actively transcribed gene sequences.
(C) Represents the entire complement of DNA from the organism's genome.
(D) Excludes repetitive DNA elements.

2015

**109.** What is the role of RNase H during the synthesis of the second cDNA strand?
(A) Synthesizes the RNA primers required for reverse transcriptase.
(B) Removes the poly-A tail from the mRNA template molecule.
(C) Ligates the newly synthesized DNA fragments together.
(D) Degrades RNA template, creating primers for DNA Pol I.

2013

**110.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): PCR-based screening of libraries is significantly faster than traditional hybridization-based screening.
Reason (R): PCR-based screening can be performed on pools of library clones, reducing the total number of assays required to identify a positive clone.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.

NODIA

2019

**184.** In hybridoma production, the fusion of B-cells and myeloma cells is an inefficient process. What is the approximate frequency of successful hybridoma formation?
(A) 1 in $10^1$ cells
(B) 1 in $10^2$ cells
(C) 1 in $10^5$ cells
(D) 1 in $10^7$ cells

2007

**185.** A sandwich ELISA involves coating the microtiter plate with:
(A) The target antigen immobilized on the surface.
(B) A capture antibody specific to the target antigen.
(C) An enzyme-linked secondary antibody for detection.
(D) The chromogenic substrate for enzymatic reaction.

2011

**186.** The standard curve in a Radioimmunoassay (RIA) is constructed by plotting:
(A) The fluorescence intensity against known concentrations.
(B) Ratio of bound to free vs. unlabeled antigen.
(C) The specific activity of the isotope against time.
(D) The absorbance at 450 nm against serial dilutions.

2003

**187.** What dictates the spatial resolution of a FISH assay on metaphase chromosomes?
(A) The degree of chromatin condensation
(B) The length of the fluorescent probe used
(C) The intensity of the UV light source
(D) The concentration of formamide in the buffer

2013

**188.** Differential centrifugation is typically performed by subjecting a cell homogenate to:
(A) A continuous density gradient of sucrose
(B) A constant low-speed centrifugation for an extended period
(C) Successively increasing centrifugal forces and times
(D) An electrical field during centrifugation

2021

**189.** In gel filtration chromatography, molecules larger than the exclusion limit of the matrix will:
(A) Bind irreversibly to the matrix
(B) Elute last, after the internal volume
(C) Be degraded by the matrix
(D) Elute in the void volume

2014

**190.** Determine the correct arrangement of operations for isolating mRNA using an oligo-dT column:
(A) Equilibrate column in high salt → Apply total RNA in high salt → Wash in high salt → Elute mRNA in low salt/water
(B) Apply total RNA in low salt → Equilibrate column in high salt → Wash in low salt → Elute mRNA in high salt
(C) Equilibrate column in low salt → Apply total RNA in low salt → Wash in high salt → Elute mRNA in high salt
(D) Apply total RNA in water → Wash in water → Elute mRNA in high salt

2023

**191.** During genomic DNA extraction, proteinase K is commonly included in the lysis buffer. What makes proteinase K particularly advantageous for this application?
(A) It requires a specific DNA sequence to bind to.
(B) It is highly active in the presence of SDS and EDTA.
(C) It specifically targets and degrades only histones.
(D) It renatures the DNA backbone after lysis.

2002

**192.** During the alkaline lysis method of plasmid DNA isolation, the pH is raised to approximately 12.0-12.5. At this pH, which of the following occurs?
(A) Only genomic DNA denatures; plasmid DNA remains perfectly double-stranded.
(B) Proteins remain folded while DNA denatures.
(C) RNA degrades into single nucleotides while DNA precipitates.
(D) Both genomic and plasmid DNA denature into single strands.

2025

**193.** Why must the tissue be flash-frozen in liquid nitrogen and physically ground to a fine powder during RNA extraction from plant tissues?
(A) Deactivate RNases and physically disrupt cell wall.
(B) To precipitate the RNA prior to any buffer addition.
(C) To crosslink RNA to cellular proteins permanently.
(D) To ensure the extraction buffer remains at low temp.

2023

**246.** Which fundamental principle differentiates absolute quantification in qPCR from relative quantification?
(A) Absolute quantification uses SYBR Green, while relative quantification uses TaqMan probes.
(B) Absolute quantification requires the use of a reference/housekeeping gene for normalization.
(C) Absolute quantification requires a serially diluted standard curve of known concentration.
(D) Absolute quantification can only be performed on a genomic DNA template.

2016

**247.** Linkers used in cDNA library construction are essentially:
(A) Long stretches of poly-A nucleotides for priming.
(B) Enzymes that ligate blunt ends of DNA molecules.
(C) Radioactive probes used to identify specific clones.
(D) Short double-stranded oligonucleotides with restriction site.

2018

**248.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): PacBio SMRT sequencing is highly effective for de novo genome assembly and resolving highly repetitive genomic regions.
Reason (R): SMRT sequencing generates extremely long read lengths (often >10 kb) that can span across long repetitive sequences.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**249.** Which of the following is an advantage of using a fluorescent label in FISH instead of a radioactive label (as used in traditional in situ hybridization)?
(A) Radioactive labels provide sharper spatial resolution on chromosomes.
(B) Safer to handle, longer shelf life, allow multiplexing.
(C) Fluorescent labels require simpler equipment like a light microscope.
(D) Radioactive labels are entirely immune to all background noise.

2003

**250.** Based on the following statements concerning analytical ultracentrifugation, select the correct option:
I. It requires the use of extremely small sample volumes compared to preparative ultracentrifugation.
II. It cannot be used to determine the exact molecular weight of a protein complex.
III. Sedimentation velocity experiments measure the rate at which molecules move in response to centrifugal force.
IV. The rotor is equipped with specialized optical windows.
V. It is primarily used to purify large batches of whole cells.
Which of the above statements is/are correct?
(A) I, III and IV
(B) II, IV and V
(C) I, II and III
(D) III, IV and V

**********

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(A) Bacterial Artificial Chromosome (BAC).
(B) Lambda phage genomic insertion vector.
(C) Cosmid hybrid cloning vector system.
(D) Plasmid small-scale cloning vector.

2011

**204.** During cDNA library construction, mRNA must be isolated from the total RNA pool. What percentage of total cellular RNA does mRNA typically represent?
(A) 15-20%
(B) 50-60%
(C) 1-5%
(D) 80-90%

2014

**205.** In a blue-white screening system, white colonies indicate:
(A) Non-recombinant plasmids with an intact lacZ' gene.
(B) Bacterial cells that have failed to take up any plasmid.
(C) Plasmids that lack a functional antibiotic resistance.
(D) Recombinant plasmids where lacZ' is disrupted by insert.

2019

**206.** In Sanger sequencing, the ratio of normal dNTPs to terminating ddNTPs in the reaction mix must be:
(A) 1:1, meaning equal amounts of both types are used.
(B) Highly skewed towards ddNTPs to ensure termination occurs.
(C) Skewed towards dNTPs, to allow extension before termination.
(D) Entirely dependent on the GC content of the template DNA.

2014

**207.** Ion Torrent sequencing technology differs from Illumina sequencing primarily because it detects:
(A) The emission of visible light
(B) The release of a hydrogen ion ($H^+$) resulting in a pH change
(C) The release of pyrophosphate via luciferase
(D) The molecular weight of the extended DNA strand

2012

**208.** The secondary antibody in an Indirect ELISA is specific for:
(A) The target antigen
(B) The enzyme conjugated to the plate
(C) The blocking buffer proteins
(D) The Fc region of the primary antibody

2025

**209.** What is a key disadvantage of utilizing polyclonal antibodies compared to monoclonal antibodies in diagnostic assays?
(A) Highly susceptible to cross-reactivity and variation.
(B) They are significantly more expensive to produce.
(C) They bind to only a single epitope, reducing sensitivity.
(D) They cannot be conjugated to enzymes or fluorophores.

2025

**210.** Given below are two statements, one is Assertion (A) and the other is Reason (R). Based on these, choose the most appropriate answer from the options provided:
Assertion (A): Competitive ELISA is specifically advantageous for detecting small antigens or haptens.
Reason (R): Small antigens typically possess only a single epitope, preventing the simultaneous binding of two antibodies required in a Sandwich ELISA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**211.** In RIA, increasing the concentration of unlabeled target antigen in the patient sample will result in:
(A) Less radiolabeled antigen binding to the antibody
(B) More radiolabeled antigen binding to the antibody
(C) An increase in total antibody concentration
(D) Precipitation of the unbound radiolabel

NODIA

2024

**212.** Which FISH technique utilizes multiple distinct fluorophores to paint the entire karyotype of a cell?
(A) Locus-specific fluorescent in situ hybridization.
(B) Centromeric fluorescent in situ hybridization.
(C) Chromogenic In Situ Hybridization (CISH) technique.
(D) Spectral Karyotyping or Multiplex FISH.

purely based on their molecular weight.
IV. The Svedberg unit is a measure of time ( $10^{\{-13\}}$ seconds).
V. Cesium chloride is commonly used to create density gradients for DNA separation.

Which of the above statements is/are INCORRECT?
(A) I and II
(B) III only
(C) III and IV
(D) IV and V

2024

**231.** Which feature makes the Sandwich ELISA generally more specific than an Indirect ELISA?
(A) It relies on a single monoclonal antibody for both capture.
(B) It does not require a blocking step to be performed.
(C) It uses a competitive substrate that amplifies the signal.
(D) Antigen must possess at least two distinct epitopes.

2001

**232.** What is the fundamental biological limitation of generating monoclonal antibodies using standard mouse hybridoma technology for human therapeutic use?
(A) Recognized as foreign, triggering a HAMA response.
(B) They are significantly too expensive to scale up.
(C) They cannot be conjugated to standard drugs or toxins.
(D) They possess a very short half-life in typical in vitro.

2019

**233.** FISH utilizes fluorescent probes to identify target sequences. Why must the probe DNA be significantly shorter than the target chromosome sequence?
(A) To prevent the probe from hybridizing to itself.
(B) Ensure penetration of membranes during hybridization.
(C) To allow the DAPI stain to bind to the remaining DNA.
(D) To increase the melting temperature of the DNA probe.

2021

**234.** Gel filtration media consist of porous beads. A molecule that is smaller than the pore size of the beads will:
(A) Elute immediately in the void volume.
(B) Enter the pores, traversing a longer path, and elute later.
(C) Bind permanently to the inside of the pores via hydrophobic interactions.
(D) Be excluded from the column entirely.

2009

**235.** The fundamental equation relating the melting temperature ($T_m$) of DNA to its base composition implies that $T_m$:
(A) Increases linearly with the percentage of G-C pairs
(B) Increases linearly with the percentage of A-T pairs
(C) Is independent of sequence composition
(D) Decreases logarithmically with the length of the fragment

2016

**236.** Phenol used in nucleic acid extraction must be handled with extreme care because it:
(A) Is highly explosive when exposed to standard air.
(B) Causes severe chemical burns and acts as a potent toxin.
(C) Rapidly degrades plastic microcentrifuge tubes used.
(D) Emits $\alpha$ radiation under laboratory conditions.

2008

**237.** The principle of using a silica column to isolate genomic DNA relies on the formation of what type of bridges between the silica surface and the DNA backbone in the presence of chaotropic salts?
(A) Disulfide bridges formed between protein residues.
(B) Covalent phosphodiester bridges with the silica.
(C) Peptide bridges formed during the lysis process.
(D) Cation bridges (utilizing sodium or guanidinium ions).

2022

**238.** Based on the chemistries of Next Generation Sequencing (NGS), match List I with List II:
List I: (NGS platforms)
P. Illumina
Q. Ion Torrent

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2006

**222.** The term "electroblotting" in Western blotting refers to:
(A) Using electricity to denature the proteins prior to running.
(B) Detecting the specific proteins using electrochemiluminescence.
(C) Electric field to drive proteins out of gel onto membrane.
(D) Applying an electric current to block the membrane sites.

2023

**223.** What is the fundamental cause of a "primer dimer" artifact in PCR?
(A) The polymerase synthesizing DNA without any template.
(B) Primers have partial complementarity, allowing hybridization.
(C) The annealing temperature being set too high for PCR.
(D) The template DNA forming a stable secondary structure.

2007

**224.** Based on the following statements concerning genomic library construction, select the correct option:
I. Libraries are ideally constructed using completely digested genomic DNA to ensure uniformity.
II. Vectors with high carrying capacity, like BACs, are preferred for eukaryotic libraries.
III. T4 DNA ligase is required to link the genomic fragments to the vector.
IV. To prevent self-ligation, the vector is often treated with alkaline phosphatase.
V. Genomic libraries contain both intron and exon sequences.
Which of the above statements is/are correct?
(A) II, III, IV and V
(B) I, II, III and V
(C) I, III, IV and V
(D) II, III and IV

2023

**225.** A cDNA library prepared from liver tissue will differ from a cDNA library prepared from brain tissue of the same organism primarily in:
(A) The types and relative abundances of the represented cloned sequences
(B) The genetic code used by the transcripts
(C) The presence of introns in the liver library but not the brain library
(D) The method used to synthesize the second DNA strand

2001

**226.** In immunological screening, what is the role of IPTG (Isopropyl β-D-1-thiogalactopyranoside)?
(A) It provides a nutrient source for the bacteria.
(B) It acts as the chromogenic substrate that produces a color change.
(C) It induces the expression of the cloned gene by de-repressing the lac promoter.
(D) It lysis the bacterial cells, releasing the protein.

NODIA

2005

**227.** A key requirement for an ideal cloning vector to be used in library construction is:
(A) Multiple unique restriction sites located within a selectable marker gene
(B) The absence of an origin of replication
(C) A large size, typically over 100 kb, for basic plasmid vectors
(D) Sensitivity to standard bacterial restriction enzymes

2005

**228.** Which NGS technique is characterized by detecting the release of a pyrophosphate molecule via a light-emitting cascade?
(A) Reversible dye-terminator sequencing
(B) SMRT sequencing
(C) Ion semiconductor sequencing
(D) Pyrosequencing

2004

**229.** What is the primary role of the "feeder layer" of cells often used during the cloning of hybridoma cells by limiting dilution?
(A) To provide essential growth factors and support cell viability at low densities.
(B) To selectively kill any remaining unfused B-cells.
(C) To act as a physical barrier to prevent cell-to-cell contact.
(D) To increase the frequency of somatic hypermutation in the clones.

2019

**230.** Based on the following statements regarding ultracentrifugation, select the correct option:
I. Analytical ultracentrifuges are equipped with optical systems to observe sedimentation in real time.
II. In rate-zonal centrifugation, the sample is layered on top of a continuous density gradient.
III. Isopycnic centrifugation separates particles

2013

**213.** When separating organelles by differential centrifugation, which component typically pellets first at the lowest speed (e.g., 600 x g for 10 minutes)?
(A) Microsomes and ribosomes
(B) Whole cells and nuclei
(C) Mitochondia and lysosomes
(D) Soluble cytosolic proteins

2006

**214.** In affinity chromatography, the specific interaction between the matrix and the target protein is primarily governed by:
(A) Covalent bonds formed between the matrix and protein.
(B) General electrostatic attractions between the charges.
(C) Biological recognition (e.g., enzyme-substrate).
(D) Large differences in the molecular weight of proteins.

2003

**215.** What is the fundamental principle behind using the A260/280 ratio to estimate nucleic acid purity?
(A) Nucleic acids absorb maximally at 280 nm, while proteins absorb at 260 nm.
(B) The ratio measures the exact molecular weight of contaminating proteins.
(C) It quantifies the total amount of phenol remaining in the sample.
(D) NA absorb at 260 nm, while proteins absorb at 280 nm.

2002

**216.** During the precipitation of genomic DNA, ethanol is preferred over isopropanol when:
(A) The pellet needs to be washed and dried quickly.
(B) The starting volume is very large and needs to be minimized.
(C) A higher coprecipitation of salts is desired.
(D) The extraction buffer contains high amounts of SDS.

2005

**217.** The alkaline lysis method exploits the difference in renaturation capabilities between plasmid and genomic DNA. After neutralization, why does plasmid DNA renature correctly while genomic DNA tangles and precipitates?
(A) Genomic DNA lacks the sequence complementarity to renature.
(B) Plasmid DNA circularity keeps complementary strands linked.
(C) Plasmid DNA relies on unique proteins that protect it from heat.
(D) Genomic DNA contains a significantly higher percentage of AT.

2011

**218.** When isolating total RNA, the use of DEPC (diethyl pyrocarbonate) treated water is strictly required to:
(A) Inactivate RNases by modifying amino acid residues.
(B) Deactivate trace DNase activity in the solution.
(C) Precipitate the RNA out of the aqueous solution.
(D) Increase the total solubility of the RNA pellet.

2021

**219.** The molar extinction coefficient ($\varepsilon$) for double-stranded DNA at 260 nm is generally accepted to be:
(A) 0.020 $(\mu g/mL)^{-1}$ $cm^{-1}$
(B) 1.0 $(\mu g/mL)^{-1}$ $cm^{-1}$
(C) 33 $(\mu g/mL)^{-1}$ $cm^{-1}$
(D) 50 $(\mu g/mL)^{-1}$ $cm^{-1}$

2004

**220.** Based on the principles of chromatography, match List I with List II:
List I: (Chromatography techniques)
P. Gel filtration chromatography
Q. Affinity chromatography
R. Anion-exchange chromatography
S. Cation-exchange chromatography
List II: (Separation principles)
1. Binds negatively charged molecules
2. Separates based on molecular size and shape
3. Binds positively charged molecules
4. Separates based on specific reversible biological interactions
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-3, S-1
(C) P-2, Q-4, R-3, S-1
(D) P-1, Q-3, R-2, S-4

2023

**221.** In Native PAGE, the electrophoretic mobility of a protein is determined by:
(A) Its molecular weight alone
(B) Its isoelectric point alone
(C) Its sequence of amino acids exclusively
(D) Its charge-to-mass ratio and its physical conformation

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R. PacBio SMRT
S. Pyrosequencing

List II: (Detection chemistries)

1. Real-time detection of fluorescent pulses in zero-mode waveguides
2. Detection of pH change via semiconductor sensors
3. Detection of light produced by a luciferase cascade
4. Imaging of reversible fluorescent dye-terminators

(A) P-4, Q-2, R-1, S-3
(B) P-1, Q-3, R-2, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-2, Q-4, R-3, S-1

2005

**239.** An investigator extracts RNA and measures the $\frac{A_{260}}{A_{280}}$ ratio to be 1.95. This indicates:
(A) The RNA is highly contaminated with genomic DNA.
(B) RNA is highly pure and largely free of protein.
(C) The RNA has degraded completely into nucleotides.
(D) The sample contains a high amount of phenol carryover.

2021

**240.** Based on the following statements concerning plasmid isolation, select the correct option:

I. RNase A is added to ensure RNA does not copurify with the plasmid DNA.
II. Solution II contains SDS and NaOH.
III. Gentle inversion is required during lysis to prevent shearing of genomic DNA.

Which of the above statements is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2025

**241.** How does the intercalation of ethidium bromide affect the electrophoretic mobility of double-stranded DNA?
(A) It increases the mobility by increasing the net negative charge density.
(B) It decreases the mobility by increasing the mass and reducing the net negative charge.
(C) It has no effect on mobility as it is a neutral molecule.
(D) It causes the DNA to migrate towards the negative electrode.

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2006

**242.** In phage display technology, the protein of interest is usually fused to which part of the bacteriophage?
(A) The genomic DNA inside the capsid.
(B) The tail fibers responsible for attachment.
(C) A coat protein displayed on the exterior surface.
(D) The internal lysozyme used for bacterial lysis.

2002

**243.** In SDS-PAGE, if the stacking gel is omitted, what is the most likely result?
(A) Bands smeared and diffuse due to lack of pre-concentration.
(B) The proteins will fail to enter the resolving gel entirely.
(C) The proteins will resolve based on native charge rather than size.
(D) The gel will melt due to excessive heat generation during electrophoresis.

2010

**244.** Which factor is the most significant determinant of the stringency during the hybridization wash step in Southern blotting?
(A) The concentration of the DNA polymerase.
(B) The temperature and salt concentration of the wash buffer.
(C) The thickness of the agarose gel used for separation.
(D) The intensity of the UV light during crosslinking.

2019

**245.** Based on the following statements regarding PCR primer design, select the correct option:

I. Primers should ideally have a GC content of 40-60%.
II. The melting temperatures (Tm) of the forward and reverse primers should differ by at least 10°C.
III. Runs of three or more G or C residues at the 3' end should be avoided to prevent non-specific annealing.
IV. Primer length is typically 18-25 nucleotides.

Which of the above statements is/are INCORRECT?
(A) I and IV
(B) II only
(C) III and IV
(D) I and II

2007

**194.** A researcher wants to quantify a very dilute DNA sample accurately. Which method provides superior sensitivity over standard UV spectrophotometry?
(A) Visual estimation on an agarose gel without a standard
(B) Absorbance measurement at 280 nm
(C) Fluorometry using PicoGreen dye
(D) Measuring the A260/230 ratio

2008

**195.** In agarose gel electrophoresis, what is the primary consequence of using water instead of an appropriate buffer (like TAE or TBE)?
(A) Gel fails to conduct current, DNA will not migrate.
(B) The DNA will migrate much faster due to lower resistance.
(C) The DNA will immediately degrade in the water solution.
(D) The ethidium bromide will fail to intercalate correctly.

2006

**196.** Order the steps of a typical PCR cycle:
I. Extension
II. Annealing
III. Denaturation
(A) I, II, III
(B) III, II, I
(C) II, I, III
(D) III, I, II

2012

**197.** Southern blotting is considered a technique of historical importance, largely superseded by which modern technique for detecting specific DNA sequences?
(A) Flow cytometry
(B) Polymerase Chain Reaction (PCR)
(C) Western blotting
(D) Ultracentrifugation

2023

**198.** Glyoxal denaturation of RNA is preferred in some Northern blotting protocols because, unlike formaldehyde:
(A) It permanently crosslinks the RNA to the gel.
(B) It acts as a tracking dye during electrophoresis.
(C) It does not require a fume hood and is less toxic.
(D) It increases the speed of capillary transfer.

2006

**199.** The most common blocking agents used in Western blotting to prevent non-specific binding are:
(A) Detergents like SDS or Triton X-100 solutions.
(B) Alcohols such as methanol or ethanol solutions.
(C) Proteases like Proteinase K or Trypsin enzymes.
(D) Bovine serum albumin (BSA) or non-fat dried milk.

2024

**200.** Based on the following statements regarding quantitative PCR (qPCR), select the correct option:
I. The threshold line is set in the plateau phase of the amplification curve.
II. A lower $C_t$ value indicates a higher initial concentration of target DNA.
III. TaqMan probes contain both a fluorophore and a quencher molecule.
IV. SYBR Green only emits strong fluorescence when bound to double-stranded DNA.
Which of the above statements is/are INCORRECT?
(A) I only
(B) II and III
(C) I and IV
(D) III and IV

2013

**201.** Which enzyme is used in RT-PCR to specifically degrade the RNA template after the first strand of cDNA has been synthesized?
(A) RNase A
(B) RNase H
(C) DNase I
(D) S1 Nuclease

2012

**202.** A standard qPCR amplification curve is typically plotted on which type of axes?
(A) Linear fluorescence vs. logarithmic cycle number
(B) Logarithmic fluorescence vs. linear cycle number
(C) Linear fluorescence vs. linear cycle number
(D) Logarithmic fluorescence vs. logarithmic cycle number

2015

**203.** Which vector allows for the largest insert size, making it most suitable for eukaryotic genomic libraries?

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# ANSWERS

**1.** (A) Disruption of hydrogen bonds and unstacking of nitrogenous bases
Denaturation of double-stranded DNA into single strands leads to an increase in UV light absorption at 260 nm. This physical phenomenon occurs because the nitrogenous bases, which were previously stacked and hydrogen-bonded within the helix, become exposed. The unstacking of these aromatic rings reduces the electronic shielding, thereby significantly increasing their molar extinction coefficient.

**2.** (C) Cetyltrimethylammonium bromide
Cetyltrimethylammonium bromide is a cationic detergent that plays a dual role during the extraction of nucleic acids from plant tissues. It effectively disrupts the lipid bilayer of the cell membrane and the nuclear envelope, facilitating cell lysis. Furthermore, it denatures proteins and forms complexes with polysaccharides, which helps in separating these contaminants from the DNA molecules.

**3.** (A) It lowers the pH, causing genomic DNA and proteins to precipitate.
Adding potassium acetate to the alkaline lysate serves to neutralize the high pH caused by sodium hydroxide. This rapid neutralization causes the large, denatured genomic DNA and cellular proteins to form an insoluble white precipitate. The potassium ions also interact with the detergent to form an insoluble complex, while the small plasmids remain in solution.

**4.** (D) The acidic pH of the extraction medium
In the guanidinium thiocyanate-phenol-chloroform method, maintaining an acidic pH is crucial for selective extraction. Under these conditions, DNA remains relatively non-polar and partitions into the organic phenol-chloroform phase or the interphase. Conversely, RNA remains highly polar and stays dissolved in the upper aqueous phase, allowing for its specific recovery from the complex cellular mixture during centrifugation.

**5.** (B) 25 μg/mL
The concentration of double-stranded DNA can be determined using its known extinction coefficient, where an absorbance of one at 260 nanometers corresponds to approximately 50 micrograms per milliliter. Given a measured absorbance of 0.5 in a standard path length cuvette, the concentration is calculated by multiplying this value by the conversion factor, resulting in twenty-five micrograms per milliliter.

**6.** (C) They bind directly to DNA to induce fluorescence under UV light.
Loading dyes serve purely operational purposes in electrophoresis, such as increasing sample density to ensure DNA sinks into the wells. They also provide visible migration markers to monitor progress. However, unlike intercalating agents such as ethidium bromide, these dyes do not bind to the DNA molecules themselves nor do they possess the ability to induce fluorescence.

**7.** (D) To concentrate protein samples into a narrow band before resolution
The stacking gel is designed with a lower acrylamide concentration and a specific pH to facilitate the concentration of protein samples. By utilizing the isotachophoresis principle, proteins are compressed into a very thin, highly concentrated starting zone at the interface with the resolving gel. This ensures that all proteins begin their separation by molecular weight simultaneously for sharp resolution.

**8.** (A) To fragment DNA, facilitating easier transfer to the membrane
Large DNA fragments migrate very slowly out of an agarose gel during the transfer process. Treating the gel with dilute hydrochloric acid induces depurination, which subsequently leads to the cleavage of the phosphodiester backbone at those sites. This fragmentation reduces the size of the DNA molecules, significantly increasing the efficiency and speed of their transfer onto the membrane.

**9.** (B) To prevent the formation of secondary structures in RNA
RNA molecules naturally form complex secondary and tertiary structures through internal base pairing, which can interfere with their migration according to size. Formaldehyde

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acts as a denaturing agent within the agarose gel, forming adducts with the bases to prevent these interactions. This ensures that the RNA remains in a linear, unfolded state, allowing for accurate size-based separation.

**10.** (A) Both A and R are true and R is the correct explanation of A.
Polyclonal antibodies are produced by multiple B-cell clones, resulting in a mixture that recognizes various epitopes on a single antigen. This lack of specificity often leads to cross-reactivity with unintended targets, making them less ideal for sensitive therapeutic uses. Additionally, the inherent biological variation between different production batches compromises the reproducibility required for standardized clinical or pharmaceutical applications.

**11.** (A) Using an annealing temperature significantly below the primer $T_m$
The annealing temperature is a critical parameter that determines the specificity of primer binding to the template DNA. When the temperature is set significantly below the calculated melting point of the primers, it allows for stable base pairing at mismatched or partially complementary sequences. This leads to the amplification of unintended genomic regions, resulting in multiple off-target product bands.

**12.** (C) Oligo-dT priming
Eukaryotic messenger RNA molecules are characterized by a polyadenylated tail at their three-prime end. Utilizing oligo-dT primers, which consist of a short string of thymidine residues, allows for the specific hybridization to this tail. This strategy ensures that cDNA synthesis is initiated only from mRNA templates, effectively excluding the high abundance of ribosomal RNA lacking such structures.

**13.** (D) It is inversely proportional to the logarithm of the template concentration.
The cycle threshold represents the point during real-time PCR where the fluorescent signal exceeds the background noise. This value is determined by the initial number of template molecules present in the reaction. Since amplification occurs exponentially, a higher starting concentration requires fewer cycles to reach the threshold. Therefore, the cycle threshold value decreases linearly as the logarithm of the concentration increases.

**14.** (C) Partial digestion using a frequent-cutting restriction enzyme
Generating a representative genomic library requires the production of overlapping DNA fragments of a suitable size for the vector. Frequent-cutting restriction enzymes recognize four-base pair sequences and occur often in the genome. By performing a partial digestion, researchers can ensure that every part of the genome is represented in various fragments, preventing the loss of genes containing internal restriction sites.

**15.** (B) To provide cohesive ends for insertion into a cloning vector
Synthesized double-stranded cDNA typically lacks the specific sticky ends required for efficient ligation into a cloning vector. Linkers are short, chemically synthesized oligonucleotides containing internal restriction sites. Once ligated to the blunt-ended cDNA and digested with the appropriate enzyme, they provide the necessary overhanging cohesive ends for stable insertion into the chosen plasmid or bacteriophage vector system.

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**16.** (D) Plaque hybridization screens bacteriophage libraries, while colony hybridization screens plasmid libraries.
Both techniques involve transferring biological material from a culture plate to a membrane for screening with a labeled probe. Colony hybridization is specifically used to identify bacterial colonies harboring recombinant plasmids. In contrast, plaque hybridization is employed to screen clear zones on a bacterial lawn, which represent areas of lysis caused by recombinant bacteriophage particles carrying the target sequence.

**17.** (A) 3'-hydroxyl
DNA polymerase requires a free three-prime hydroxyl group on the preceding nucleotide to catalyze the formation of a phosphodiester bond with the incoming deoxynucleotide. Dideoxynucleotides are structural analogs that lack this specific hydroxyl group. When a dideoxynucleotide is incorporated into the growing DNA strand, it acts as a permanent terminator because no further nucleotides can be covalently attached.

**18.** (C) Ion Torrent sequencing
Ion Torrent technology utilizes a semiconductor chip to detect chemical changes during DNA synthesis. When a DNA polymerase incorporates a nucleotide into the growing strand, a hydrogen ion is naturally released as a byproduct. This release causes a localized change in the pH of the solution, which the sensors on the chip convert into a measurable electronic signal for sequencing.

**19.** (A) They lack the enzyme hypoxanthine-guanine phosphoribosyltransferase.
HAT medium contains aminopterin, which blocks the de novo pathway for purine synthesis. Cells must rely on the salvage pathway, which requires the enzyme hypoxanthine-guanine phosphoribosyltransferase. Myeloma cells used for fusion are intentionally selected for a deficiency in this enzyme. Consequently, any unfused myeloma cells cannot produce nucleotides in the presence of aminopterin and eventually perish.

**20.** (A) P-2, Q-4, R-1, S-3
In direct ELISA, a labeled primary antibody binds the antigen. Indirect ELISA uses an unlabeled primary antibody followed by a labeled secondary antibody. Sandwich ELISA captures the target antigen between two specific antibodies, providing high specificity. Competitive ELISA measures the reduction in signal when sample antigen competes with labeled antigen, meaning the highest signal corresponds to the lowest concentration.

**21.** (B) Radioactivity is inversely proportional to patient antigen concentration.
Radioimmunoassay is based on the principle of competitive binding between a fixed amount of radiolabeled antigen and a variable amount of unlabeled patient antigen for limited antibody binding sites. As the concentration of the unlabeled antigen in the patient sample increases, it displaces more of the radiolabeled version. Consequently, the radioactivity measured in the bound fraction decreases proportionally.

**22.** (C) Denaturation
To allow a fluorescently labeled probe to hybridize with its specific target sequence on a chromosome, the double-stranded DNA must first be separated into single strands. This denaturation step is typically achieved using heat or chemical agents like formamide. Once the strands are separated, the probe can form base pairs with its complementary sequence during the subsequent incubation.

**23.** (C) Sedimentation velocity per unit of centrifugal force
The Svedberg unit is a non-metric physical unit used to quantify the rate at which a particle sediments during ultracentrifugation. It is defined as the sedimentation velocity of the particle divided by the applied centrifugal acceleration. This value depends on several physical properties, including the mass, density, and hydrodynamic shape of the molecule or ribosomal complex being analyzed.

**24.** (A) Nickel-NTA agarose
Nickel-NTA agarose is a specialized matrix used for the isolation of proteins that have been engineered with a polyhistidine tag. The histidine residues form strong coordinate bonds with the nickel ions immobilized on the agarose beads. This high-affinity biochemical interaction allows for the selective purification of the target protein from a complex mixture of other cellular proteins and contaminants.

**25.** (B) $\frac{A_{260}}{A_{280}}$ ratio of 1.2
Purified nucleic acids are routinely assessed using the ratio of absorbance at 260 nanometers to 280 nanometers. Pure DNA typically exhibits a ratio between 1.8 and 2.0. A significantly lower value, such as 1.2, indicates the presence of aromatic amino acids from protein contamination, as proteins absorb light strongly at the 280 nanometer wavelength due to tryptophan and tyrosine.

**26.** (B) Phenol
Phenol is a powerful organic solvent used to separate proteins from nucleic acids during extraction. It acts as a protein denaturant by disrupting the internal folding of the molecules, which exposes their hydrophobic interiors. These denatured proteins then partition into the organic phenol phase or aggregate at the interphase, while the hydrophilic DNA remains dissolved in the aqueous phase.

**27.** (A) Binding of DNA to silica in the presence of high concentrations of chaotropic salts
Silica-based purification relies on the ability of nucleic acids to bind to the silica surface

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measurable signal. This allows for the detection of minute quantities of hormones or drugs in clinical samples.

**46.** (C) Stronger signal by covering a large contiguous chromosomal region.
Bacterial artificial chromosome probes are significantly larger than oligonucleotide probes, often spanning hundreds of kilobases. Because of their size, they can incorporate a much higher number of fluorescent molecules. When hybridized to a chromosome, these large probes cover a substantial contiguous region, producing a much brighter and more robust signal that is easier to visualize and localize during analysis.

**47.** (B) Buoyant density
When DNA is centrifuged at high speeds in a concentrated solution of cesium chloride, a stable density gradient is formed. The DNA molecules will migrate through the gradient until they reach a position where the density of the surrounding solution is exactly equal to their own buoyant density. This allows for the separation of DNA based on factors such as GC content or radioactive labeling.

**48.** (D) Molecular size and shape
Gel filtration, also known as size-exclusion chromatography, utilizes a column packed with porous beads. Separation is achieved as molecules pass through the column; larger molecules that cannot enter the pores elute quickly in the void volume. Smaller molecules enter the pores and take a longer, more circuitous path, causing them to elute later. This separates proteins based on their hydrodynamic volume.

**49.** (A) $T_m = 4(G+C)+2(A+T)$
For short oligonucleotide probes, typically between fourteen and twenty base pairs in length, the melting temperature can be approximated using the Wallace rule. This formula assigns four degrees Celsius for every guanine-cytosine base pair, which contains three hydrogen bonds, and two degrees Celsius for every adenine-thymine base pair, which contains only two hydrogen bonds, providing a quick estimate of stability.

**50.** (A) I, II and IV
Guanidinium thiocyanate is a powerful chaotropic agent that denatures proteins, including RNases, to protect the RNA. Chloroform is essential for separating the mixture into aqueous and organic phases. Isopropanol is used to precipitate the RNA from the resulting aqueous phase. However, statement III is incorrect because under acidic conditions, RNA partitions into the aqueous phase while DNA goes to the organic phase.

**51.** (A) Genomic DNA is linear while plasmid DNA is circular and supercoiled.
During the denaturation step of alkaline lysis, both genomic and plasmid DNA become single-stranded. However, the circular strands of plasmid DNA remain physically interlinked. Upon rapid neutralization, these interlinked plasmid strands can easily and correctly renature. In contrast, the much larger, linear genomic DNA strands cannot re-align properly and instead form a tangled, insoluble mass that precipitates with the cellular proteins.

**52.** (D) Acidic phenol causes DNA to partition into the organic phase.
The pH of phenol is a critical factor in determining the partitioning of nucleic acids between phases. When phenol is equilibrated to an alkaline pH of 8.0, both DNA and RNA remain negatively charged and polar, thus staying in the aqueous phase. If the phenol is acidic, the DNA becomes less polar and partitions into the organic phase, which is useful for RNA-only extractions.

**53.** (B) Solute concentration (c)
The Beer-Lambert law is a fundamental principle in spectrophotometry that describes the linear relationship between the absorption of light and the properties of the material through which the light is traveling. It states that the absorbance is equal to the product of the molar extinction coefficient, which is specific to the substance, the path length of the light, and the molar concentration.

**54.** (C) Charge-to-mass ratio and physical shape
Native polyacrylamide gel electrophoresis is performed under non-denaturing conditions, which means the proteins retain their original three-dimensional structure and biological activity. Unlike SDS-PAGE, which separates proteins strictly by their molecular weight, the migration in a native gel is influenced by the protein's intrinsic net charge, its overall molecular mass, and its specific hydrodynamic shape or size in solution.

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RNA template within the RNA-DNA hybrid during synthesis. This can lead to premature termination of the growing cDNA strand. Mutant versions of the Moloney Murine Leukemia Virus reverse transcriptase that lack this activity are preferred for synthesizing long, full-length cDNA because they keep the template intact.

**37.** (B) Melt curve (dissociation) analysis
Non-specific dyes used in real-time PCR, such as SYBR Green, bind to any double-stranded DNA molecule present in the reaction. This includes primer dimers and unintended amplification products. A melt curve analysis, performed by gradually increasing the temperature after the run, identifies the number and melting points of the products. A single sharp peak confirms the presence of a specific target.

**38.** (C) The physical geometry of the phage head
The packaging mechanism of the lambda bacteriophage is highly sensitive to the total length of the DNA molecule. Recombinant DNA must fall within approximately seventy-eight to one hundred and five percent of the wild-type genome size to be successfully encapsulated. This physical constraint is dictated by the internal volume and structural geometry of the icosahedral phage head during assembly.

**39.** (D) RNase H, DNA Polymerase I, and DNA ligase
After the synthesis of the first cDNA strand, the RNA template must be partially removed and replaced. RNase H is used to create nicks in the RNA, leaving small fragments to act as primers. DNA polymerase I then uses these primers to synthesize the second DNA strand while simultaneously removing the remaining RNA. Finally, DNA ligase seals the resulting nicks.

**40.** (A) Immobilize cells on membrane → Lyse cells and denature DNA → Hybridize with probe → Autoradiography/Detection
The screening process begins by transferring bacterial colonies from an agar plate to a solid membrane, effectively immobilizing them. The cells on the membrane are then lysed to release their DNA, which is simultaneously denatured and fixed to the surface. This is followed by incubation with a labeled probe that hybridizes to the target sequence, which is eventually visualized through detection methods.

**41.** (B) Size using a sieving polymer matrix
In modern automated Sanger sequencing, DNA fragments are separated within a narrow glass capillary filled with a liquid sieving polymer. As an electric field is applied, the fragments migrate through the polymer matrix. Because the matrix retards larger molecules more than smaller ones, the DNA fragments are separated with single-base resolution according to their size before reaching the detector.

**42.** (D) III only
Pyrosequencing indeed utilizes luciferase to produce light from pyrophosphate, and Illumina uses reversible terminators. However, the statement regarding PacBio SMRT sequencing is incorrect. SMRT sequencing is a single-molecule technique that does not require any prior amplification of the DNA template. Instead, it monitors the activity of a single polymerase enzyme in real-time using zero-mode waveguide technology for detection.

**43.** (A) Block the de novo pathway of nucleotide synthesis.
Aminopterin is a potent folic acid antagonist that inhibits the enzyme dihydrofolate reductase. This inhibition effectively blocks the de novo biosynthetic pathway for the production of purines and pyrimidines within the cell. This metabolic blockade forces cells to utilize the alternative salvage pathway, which provides a mechanism for selecting hybrid cells that possess the necessary enzymes to bypass this inhibition.

**44.** (C) Competitive ELISA
Small molecules or haptens often lack sufficient surface area to be bound by two different antibodies at the same time, which is a requirement for the sandwich ELISA format. In such cases, a competitive ELISA is used. In this assay, the sample antigen competes with a labeled version for a limited number of antibody binding sites, allowing for the quantification of the molecule.

**45.** (D) High specific activity of the radiolabeled antigen
The sensitivity of a radioimmunoassay, which refers to the minimum amount of antigen that can be accurately detected, is primarily governed by the specific activity of the radioactive tracer. High specific activity ensures that even very low concentrations of the antigen produce a

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under specific conditions. In the presence of high concentrations of chaotropic salts, such as guanidinium hydrochloride, the hydration shell around the DNA is disrupted. This facilitates the formation of cation bridges between the negatively charged silica and the DNA phosphate backbone, allowing for efficient binding.

**28.** (C) It requires intact poly-A tails, thus missing degraded mRNA.
The primary limitation of using oligo-dT affinity chromatography is its absolute dependence on the presence of a stable poly-A tail. While this allows for the exclusion of ribosomal RNA, it also means that any messenger RNA molecules that have undergone degradation at their three-prime end will not be captured. This can lead to a biased representation of the transcriptome.

**29.** (D) Is highly specific to dsDNA and less affected by free nucleotides.
Fluorometric methods utilize dyes that exhibit a significant increase in fluorescence only upon binding to double-stranded DNA. Unlike UV spectrophotometry at 260 nanometers, which detects all aromatic rings including free nucleotides and single-stranded DNA, fluorometry is highly specific. This provides a more accurate quantification of intact double-stranded templates, especially in samples containing significant amounts of degraded nucleic acid.

**30.** (A) I and II
Higher concentrations of agarose create a denser matrix with smaller pores, which is necessary for resolving smaller DNA fragments. Buffer choice also impacts resolution, with TBE providing better separation for small fragments due to its higher buffering capacity and effect on DNA mobility. While ethidium bromide intercalates into DNA, it is a positively charged molecule that migrates towards the negative cathode.

**31.** (B) Binding of SDS at a constant ratio of 1.4 g SDS per gram of protein.
Sodium dodecyl sulfate is an anionic detergent that denatures proteins and binds to the polypeptide backbone at a nearly constant ratio. Specifically, approximately 1.4 grams of the detergent binds to every gram of protein. This massive accumulation of negative charge masks the intrinsic charges of the amino acids, ensuring that the charge-to-mass ratio is uniform across all proteins.

**32.** (D) To stabilize the single-stranded DNA for efficient capillary transfer
Following the denaturation of DNA within the agarose gel using sodium hydroxide, a neutralization buffer is applied to lower the pH. This step is essential because the high alkaline conditions can interfere with the binding of DNA to the nylon or nitrocellulose membrane. Neutralization stabilizes the single-stranded DNA state while ensuring the pH is compatible with efficient capillary transfer.

**33.** (A) Depurination with dilute HCl
Depurination using dilute hydrochloric acid is used in Southern blotting to break large DNA fragments into smaller pieces for easier transfer. This step must be omitted in Northern blotting because RNA is inherently more fragile than DNA. Treatment with acid would lead to the excessive hydrolysis and degradation of the RNA transcripts, making it impossible to detect discrete bands.

**34.** (C) High background signal will appear due to non-specific antibody binding.
Nitrocellulose and PVDF membranes are designed to bind proteins with high affinity. If the unoccupied sites on the membrane are not effectively blocked with inert proteins like those found in milk or bovine serum albumin, the primary and secondary antibodies will bind non-specifically to these open areas. This results in an overwhelming background signal that obscures the target protein bands.

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**35.** (A) Increasing the annealing temperature
The appearance of multiple off-target bands in a PCR reaction is often caused by primers binding to sequences that are only partially complementary. By increasing the annealing temperature, the stringency of the reaction is raised. This higher thermal energy prevents the stabilization of imperfectly matched primer-template duplexes, ensuring that amplification only occurs from the site where the primer is perfectly complementary.

**36.** (D) MMLV Reverse Transcriptase (RNase H minus mutant).
Standard reverse transcriptase enzymes possess an inherent RNase H activity that degrades the

**55.** (D) High pH prevents the hybridization of the DNA probe later.
The denaturation step in Southern blotting uses sodium hydroxide to make DNA single-stranded. However, hybridization with the labeled probe requires a neutral pH environment to allow stable hydrogen bonding between the probe and target. Failing to neutralize the gel after the alkali treatment leaves the environment too basic, which prevents the formation of DNA duplexes and results in no signal.

**56.** (A) III, I, II, V, IV
In a typical Western blot, proteins are first separated by SDS-PAGE (III) based on size. They are then transferred to a PVDF membrane (I) for detection. The membrane is blocked with non-fat milk (II) to prevent nonspecific antibody binding. Next, it is incubated with the primary antibody (V) specific to the target protein, followed by the secondary antibody (IV), which enables visualization of the protein signal.

**57.** (B) Oligonucleotide primers
DNA polymerases, including the heat-stable Taq polymerase, are unable to initiate the synthesis of a DNA strand from scratch. They require a pre-existing three-prime hydroxyl group to which they can add new nucleotides. Oligonucleotide primers are short, single-stranded sequences that bind to the target template, providing the specific double-stranded starting point and free hydroxyl group necessary for the polymerase.

**58.** (B) Standard curve using known concentrations of template.
To determine the exact number of copies or concentration of a target sequence in an unknown sample, absolute quantification is required. This involves creating a standard curve by performing qPCR on a serial dilution of a template with a known concentration. The cycle threshold values of the unknown samples are then compared to this curve to interpolate their starting quantities.

**59.** (A) Oligo(dT) primers
The presence of a polyadenylated tail is a unique structural feature of eukaryotic messenger RNA. During the construction of a cDNA library, this tail serves as a convenient binding site for an oligo-dT primer. This primer consists of a sequence of deoxythymidine residues that hybridize to the tail, providing the starting point for reverse transcriptase to synthesize the first DNA strand.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Large recombinant DNA molecules, such as those used in lambda phage libraries, cannot be efficiently introduced into bacterial cells using standard chemical transformation methods. In vitro packaging solves this by encapsulating the recombinant DNA into infectious phage particles. These particles then naturally inject the DNA into the host bacteria with very high efficiency, ensuring the successful creation of a comprehensive library.

**61.** (D) Represents only the transcribed sequences of a specific tissue.
A genomic library contains the entire DNA sequence of an organism, including both coding and non-coding regions like introns and promoters. In contrast, a cDNA library is constructed from messenger RNA using reverse transcriptase. Since mRNA has already undergone splicing in eukaryotic cells, the resulting cDNA library represents only the genes that were actively being transcribed in the specific tissue or cell type.

**62.** (C) A randomly generated 6-base pair RNA fragment
Effective screening of a cDNA library requires a probe that has a high degree of specificity for the target gene. Homologous sequences, degenerate oligonucleotides based on protein data, and antibodies for expression libraries are all valid tools. A randomly generated six-base pair RNA fragment is far too short and non-specific, as it would likely bind to thousands of unintended sites.

**63.** (B) A heterozygous locus or a single nucleotide polymorphism.
In an automated Sanger sequencing read, each of the four nucleotides is represented by a distinct fluorescent color. Usually, each position shows a single clean peak. If a specific location shows two overlapping peaks of roughly equal height but different colors, it suggests the presence of two different alleles at that genomic position, characteristic of a heterozygote or a single nucleotide polymorphism.

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**64.** (D) Reversible dye-terminator nucleotides
Illumina sequencing is based on a sequencing-by-synthesis approach that uses special deoxynucleotides. These nucleotides are modified with a fluorescent label and a chemical group that blocks further elongation. After incorporation and imaging of the fluorescence, the block and the dye are chemically removed, allowing the next nucleotide to be added. This cycle is repeated for hundreds of rounds to determine the sequence.

**65.** (A) Isolate single-cell clones to ensure true monoclonality.
Following the initial HAT selection, the resulting culture contains a mixture of various hybridoma cells, each producing a different antibody. To ensure the production of a truly monoclonal antibody, it is necessary to isolate a single hybridoma cell. Serial dilution, or limiting dilution, involves diluting the cell suspension so that individual wells in a plate contain only one cell, which then grows.

**66.** (C) Secondary antibody
In an indirect ELISA, the target antigen is first immobilized on the plate and then bound by an unlabeled primary antibody derived from a specific host species. The detection signal is generated by a secondary antibody that is specific for the constant region of the primary antibody. This secondary antibody is covalently conjugated to an enzyme that catalyzes a visible color change.

**67.** (A) Radiolabeled and unlabeled antigen for a specific antibody.
Radioimmunoassay is a highly sensitive technique used to quantify antigens. It operates on the principle of competition, where a fixed, known amount of a radiolabeled version of the antigen and the unlabeled antigen from a sample compete for a limited number of binding sites on a specific antibody. The amount of radioactive antigen that binds is measured to determine the sample concentration.

**68.** (D) Stringency (temperature and formamide concentration)
The hybridization step in FISH involves the annealing of a labeled DNA probe to its target chromosomal sequence. To ensure that the probe only binds to its perfectly complementary site and not to similar but non-target regions, the stringency must be carefully controlled. This is achieved by adjusting the incubation temperature and the concentration of formamide, which destabilizes weak, non-specific duplexes.

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**69.** (B) Specific gravity and mass
Differential centrifugation is a common technique used to separate various cellular components, such as nuclei, mitochondria, and ribosomes, from a cell homogenate. The separation is achieved by subjecting the mixture to a series of spins at progressively higher speeds. Heavier and denser particles with higher sedimentation rates will pellet at lower centrifugal forces, while smaller and lighter particles require higher forces.

**70.** (C) II only
Cation-exchange matrices are negatively charged and bind positive proteins, while anion-exchange matrices are positively charged. Gel filtration separates by size, with large molecules eluting first. Sephadex is a trade name for cross-linked dextran beads used primarily in gel filtration, not affinity chromatography. While it can be modified for affinity use, its primary and standard application is size-based separation.

**71.** (D) Chaotropic salts, phenol, and carbohydrates
While the $\frac{A_{260}}{A_{280}}$ ratio is used to check for protein contamination, the absorbance at 230 nanometers is used to identify other types of impurities. A low $\frac{A_{260}}{A_{230}}$ ratio, typically below 2.0, suggests the presence of residual contaminants from the extraction process. These include carbohydrates, EDTA, phenol, or chaotropic salts like guanidinium thiocyanate, which can inhibit subsequent enzymatic reactions.

**72.** (A) Decreases the dielectric constant, precipitating DNA.
DNA is a highly polar molecule due to its negatively charged phosphate backbone, which makes it soluble in water. Adding absolute ethanol to an aqueous solution containing DNA and monovalent cations decreases the dielectric constant of the solvent. This reduces the solubility of the DNA, allowing the cations to neutralize the phosphate groups and causing the DNA to precipitate out of solution.

**73.** (B) Shears genomic DNA, leading to plasmid contamination.
During the alkaline lysis procedure, the bacterial cell is lysed, and the large genomic DNA is denatured but remains very fragile. Violent

mechanical agitation, such as high-speed vortexing, can easily shear this genomic DNA into smaller fragments. These smaller pieces may then renature and copurify with the plasmid DNA, resulting in a contaminated and poor-quality plasmid preparation.

**74.** (B) Breaks disulfide bonds, irreversibly denaturing RNases.
Ribonucleases are exceptionally stable and active enzymes that can rapidly degrade RNA during the extraction process. Many RNases are held in their active conformation by multiple internal disulfide bonds. B-mercaptoethanol is a reducing agent included in lysis buffers to break these covalent bonds. This leads to the irreversible denaturation and inactivation of the RNases, thereby protecting the integrity of the RNA.

**75.** (A) Sample is free of DNA, as DNA also absorbs at 260 nm.
Both DNA and RNA contain nitrogenous bases that absorb ultraviolet light strongly at a wavelength of 260 nanometers. Therefore, UV spectrophotometry cannot distinguish between the two types of nucleic acids. When using the absorbance at 260 nanometers to calculate the concentration of an RNA sample, it is vital to ensure that all genomic DNA has been removed, usually by DNase treatment.

**76.** (C) The DNA fragments are to be extracted and recovered from the gel.
Tris-Acetate-EDTA (TAE) and Tris-Borate-EDTA (TBE) are the two most common buffers for DNA electrophoresis. While TBE has a higher buffering capacity and is better for resolving small fragments, it can interact with the agarose and inhibit subsequent enzymatic reactions. Therefore, TAE is generally preferred when the researcher intends to cut the DNA bands out of the gel for further cloning or sequencing.

**77.** (B) 300 base pairs in 1% agarose gel
Tracking dyes are added to DNA samples to provide a visible reference for the progress of electrophoresis. Bromophenol blue is a common dye that carries a negative charge and migrates toward the positive electrode. In a standard one percent agarose gel, its migration rate is roughly equivalent to that of a linear double-stranded DNA fragment of three hundred base pairs in length.

**78.** (C) Thymine residues
After the transfer of DNA fragments from an agarose gel to a nylon membrane, the DNA must be permanently fixed to prevent it from washing off during hybridization. Exposure to ultraviolet radiation induces the formation of covalent bonds between the membrane and the DNA. This reaction specifically involves the photoreaction of thymine residues in the DNA with the amino groups on the nylon surface.

**79.** (B) Form adducts with RNA bases to prevent secondary structures.
RNA is a single-stranded molecule that frequently forms internal base pairs, resulting in complex folded structures that hinder accurate separation by size. Glyoxal is a denaturing agent used to treat RNA samples prior to electrophoresis. It reacts with the amino groups of the bases, particularly guanine, to prevent hydrogen bonding. This keeps the RNA in a fully denatured, linear state throughout the run.

**80.** (A) I, II and IV
PVDF membranes are hydrophobic and must be pre-wetted in methanol to allow buffer penetration. Non-specific binding is minimized by using blocking agents like milk or BSA, and adding detergents like Tween-20 to wash buffers. Chemiluminescence is an enzymatic reaction, not UV-induced fluorescence. Nitrocellulose is actually more brittle and has lower mechanical strength than the more durable and flexible PVDF membrane.

**81.** (A) Promotes stronger hydrogen bonding at the polymerase site.
The three-prime end of a PCR primer is the most critical region because it is where the DNA polymerase begins adding nucleotides. Guanine and cytosine form three hydrogen bonds, compared to the two bonds formed by adenine and thymine. Including a GC clamp at the three-prime end ensures a more stable and specific attachment of the primer to the template at the initiation site.

**82.** (C) DNA ligase
The synthesis of the first strand of cDNA requires an RNA template, a reverse transcriptase enzyme, deoxynucleotide triphosphates as building blocks, and a primer to initiate synthesis. DNA ligase is an enzyme used to join DNA fragments by creating phosphodiester bonds. While ligase

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is essential for later steps, such as cloning into a vector, it is not involved in the actual synthesis of the first DNA strand.

**83.** (A) Binds to any double-stranded DNA, including primer dimers.
SYBR Green I is a non-specific intercalating dye that fluoresces whenever it binds to any double-stranded DNA. This is a major disadvantage because the signal it produces can come from the target sequence, primer dimers, or non-specific amplification products. TaqMan probes, conversely, use a sequence-specific fluorogenic probe, ensuring that the detected signal originates only from the specific target sequence of interest.

**84.** (A) Both A and R are true and R is the correct explanation of A.
A genomic library must contain the entire sequence of an organism's genome. If a researcher uses complete digestion, a gene containing an internal restriction site will be split into two different clones, making it difficult to study the whole gene. Partial digestion creates a collection of overlapping fragments, which ensures that every genomic region, including those with internal sites, is represented as an intact sequence.

**85.** (B) Prevent self-ligation of the vector.
During the preparation of a cloning vector, the circular DNA is cut with a restriction enzyme, leaving five-prime phosphate groups. These groups can be easily joined back together by DNA ligase, leading to high levels of non-recombinant, self-ligated vectors. Treating the vector with alkaline phosphatase removes these phosphate groups, ensuring that ligation can only occur if an insert provides the necessary phosphates.

**86.** (C) Strong prokaryotic promoter
Immunological screening is used to identify clones in an expression library that are producing a specific protein. For the bacterial host to synthesize the eukaryotic protein from the cloned cDNA, the vector must contain the necessary regulatory sequences. A strong prokaryotic promoter, located upstream of the insertion site, is essential to drive the transcription of the cDNA into messenger RNA within the bacterium.

**87.** (D) Unincorporated dye terminators and short primers.
In automated Sanger sequencing, the results are presented as a series of colored peaks. At the very beginning of the read, there is often a messy, overlapping signal known as the "dye blob." This noise is caused by residual fluorescently labeled dideoxynucleotides that were not incorporated into a DNA strand and were not completely removed during the sample cleanup process before electrophoresis.

**88.** (A) ATP sulfurylase
Pyrosequencing is a sequencing-by-synthesis method that detects the release of inorganic pyrophosphate. When a nucleotide is incorporated, the released pyrophosphate is used as a substrate by the enzyme ATP sulfurylase. This enzyme converts the pyrophosphate into adenosine triphosphate in the presence of adenosine five-prime phosphosulfate. The resulting ATP then powers the luciferase enzyme to produce a measurable flash of light.

**89.** (B) Promote fusion of B-cell and myeloma plasma membranes.
The production of hybridomas requires the fusion of two different cell types: an antibody-producing B-cell and an immortal myeloma cell. Because cell fusion is naturally a very rare event, a chemical fusogen is used to increase the frequency. Polyethylene glycol is the most common agent for this purpose; it acts by dehydrating the cell surfaces, which allows the plasma membranes to fuse.

**90.** (A) Add capture antibody → Block plate → Add sample antigen → Add detection antibody → Add substrate
A sandwich ELISA starts by coating the wells with a capture antibody. A blocking step is then performed to prevent non-specific binding. The sample containing the target antigen is added and bound by the capture antibody. After washing, a labeled detection antibody is added to bind a different epitope on the antigen. Finally, a substrate is added to produce a detectable signal.

**91.** (A) Not disrupt the equilibrium between bound and free antigen.
Radioimmunoassay depends on the equilibrium established between the antibody and the antigens. Once this state is reached, the bound fraction must be separated from the free

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fraction for measurement. It is critical that the separation technique used, such as charcoal adsorption or second-antibody precipitation, is rapid and gentle enough that it does not disturb the existing binding equilibrium between the molecules.

**92.** (C) Counterstain all DNA for context of the probe signal.
In fluorescence in situ hybridization, DAPI is a blue-fluorescent stain that binds strongly to adenine-thymine rich regions in DNA. While the labeled probe identifies a specific sequence, DAPI is used as a general counterstain for all the nuclear DNA. This provides the necessary spatial and structural context, allowing researchers to visualize the entire chromosome set and precisely locate the probe signal.

**93.** (D) Monitor sedimentation in real-time using optical systems.
While preparative ultracentrifugation is focused on the large-scale isolation and purification of biological samples, analytical ultracentrifugation is used for characterization. Analytical machines are equipped with sophisticated optical detection systems, such as absorbance or interference optics. These allow the researcher to observe and measure the sedimentation process as it happens, providing data on the molecular weight and purity of the sample.

**94.** (C) Gradually increasing the salt concentration of the phase.
In ion-exchange chromatography, proteins are bound to the charged matrix through electrostatic interactions. To elute these proteins, the strength of the binding must be overcome. This is typically achieved by passing a buffer with an increasing concentration of salt through the column. The salt ions compete with the proteins for the charged sites on the matrix, causing the proteins to be released.

**95.** (B) Approximately 30-40%
The transition of DNA from a double-stranded helix to a single-stranded denatured state is accompanied by a significant increase in UV light absorption. This hyperchromic shift is most prominent at the 260 nanometer wavelength. For a typical sample of genomic DNA, the absorbance will increase by approximately thirty to forty percent when the hydrogen bonds are completely broken and the bases are unstacked.

**96.** (D) Prevents foaming and aids in sharp phase separation.
In the standard phenol-chloroform-isoamyl alcohol mixture used for nucleic acid extraction, isoamyl alcohol serves as an anti-foaming agent. It helps to reduce the surface tension during the vigorous mixing steps, preventing the formation of stable foam. Additionally, it aids in maintaining a sharp, well-defined interface between the organic and aqueous phases after centrifugation, which facilitates the clean recovery of the DNA.

**97.** (A) It may cause irreversible denaturation of the plasmid DNA.
Sodium hydroxide is used in alkaline lysis to denature both genomic and plasmid DNA. However, the timing is critical. If the DNA is exposed to high alkalinity for too long, the strands of the plasmid can become so extensively denatured that they cannot properly renature when the pH is neutralized. This "irreversibly denatured" plasmid DNA will not be useful for subsequent enzymatic applications.

**98.** (A) P-2, Q-3, R-4, S-1
Guanidinium thiocyanate is a powerful chaotropic agent used to denature proteins and RNases. DEPC is a chemical reagent used to treat water and buffers to inactivate any residual RNase activity. Oligo-dT cellulose is an affinity matrix used for the specific isolation of eukaryotic mRNA with poly-A tails. Isopropanol is a solvent commonly used to precipitate total RNA from an aqueous solution.

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**99.** (A) Purity $= \frac{A_{260}}{A_{280}}$
The purity of a DNA sample is most commonly estimated by calculating the ratio of the absorbance measured at two different wavelengths. DNA absorbs light maximally at 260 nanometers, whereas proteins absorb light at 280 nanometers. By dividing the absorbance at 260 nanometers by the absorbance at 280 nanometers, researchers can determine if the sample is clean or contains significant protein impurities.

**100.** (B) II only
Gel loading dyes contain substances like glycerol to ensure the DNA sample sinks into the well and stays there. Ethidium bromide visualization indeed requires a UV transilluminator to excite the dye. However, the statement that DNA migrates toward the cathode is incorrect. DNA

is negatively charged due to its phosphate backbone and will always migrate toward the positive electrode, which is the anode.

**101.** (D) 15%
The resolution of proteins in SDS-PAGE is determined by the concentration of acrylamide in the resolving gel, which dictates the pore size. A higher percentage of acrylamide creates a denser matrix with smaller pores. To effectively separate and resolve very small proteins, such as those between ten and fifteen kiltons, a high concentration gel of fifteen percent is the most suitable choice.

**102.** (C) Lower background noise due to lower non-specific binding.
While nylon membranes are physically much stronger and more durable than nitrocellulose, they often carry a high positive charge that can lead to high background signals due to non-specific binding of probes. Nitrocellulose membranes, though more fragile, are frequently preferred in certain blotting applications because they tend to produce much lower levels of background noise, resulting in clearer and more distinct signals.

**103.** (D) Molecular weight
Formaldehyde is used in Northern blot electrophoresis to ensure that the separation of RNA molecules is based purely on their length. RNA is highly prone to forming complex internal secondary structures that would alter its migration through the gel matrix. By acting as a denaturant, formaldehyde keeps the RNA in a linear state, allowing for a direct correlation between migration distance and molecular weight.

**104.** (A) Horseradish peroxidase (HRP)
Western blotting often utilizes chemiluminescence for the sensitive detection of target proteins on a membrane. The most widely used enzyme for this purpose is horseradish peroxidase, which is conjugated to the secondary antibody. When a luminol-based substrate is added, the enzyme catalyzes an oxidation reaction that results in the emission of light, which can be captured on X-ray film or imaging systems.

**105.** (B) Optimal temperature for the catalytic activity of Taq.
The extension phase of a PCR cycle is when the DNA polymerase synthesizes the new DNA strand. Taq polymerase, which was originally isolated from the thermophilic bacterium Thermus aquaticus, has evolved to function at high temperatures. While it is stable at much higher temperatures, its maximum catalytic rate for incorporating nucleotides into a growing DNA chain occurs optimally at approximately seventy-two degrees Celsius.

**106.** (B) Reverse transcription of RNA into cDNA
In a two-step RT-PCR protocol, the process is divided into two separate reactions. The first step involves the reverse transcription of the starting RNA template into complementary DNA using a reverse transcriptase enzyme and appropriate primers. Once the cDNA is synthesized, an aliquot is then transferred to a second, separate reaction tube where the actual PCR amplification of the target sequence takes place.

**107.** (A) The $2^{-\Delta\Delta C_t}$ method
The two-power-negative-delta-delta-Ct method, also known as the Livak method, is the standard mathematical approach for relative quantification in real-time PCR. This method compares the cycle threshold values of a target gene in an experimental sample to a control sample. Both values are first normalized to an internal reference or housekeeping gene to account for differences in the amount of starting template material.

**108.** (C) Represents the entire complement of DNA from the organism's genome.
A genomic library is designed to contain a comprehensive collection of all the DNA sequences found within an organism's entire genome. This includes all coding genes, introns, and intergenic regions. In contrast, a chromosome-specific library is much more focused, containing only the DNA fragments derived from a single chromosome that has been isolated through techniques such as flow cytometry or microdissection.

**109.** (D) Degrades RNA template, creating primers for DNA Pol I.
During the synthesis of the second cDNA strand, RNase H plays a critical role by specifically recognizing and cleaving the RNA

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within the RNA-DNA hybrid. Rather than completely degrading the RNA, it leaves small, fragmented RNA segments attached to the DNA. These remaining RNA fragments serve as natural primers for DNA polymerase I to begin synthesizing the second, complementary DNA strand.

**110.** (A) Both A and R are true and R is the correct explanation of A.
Traditionally, screening large genomic or cDNA libraries required labor-intensive hybridization techniques. PCR-based screening is significantly faster and more efficient because it allows for the analysis of pools of clones. By testing large groups of clones in a single reaction and then narrowing down the positive pool through subsequent rounds of testing, researchers can identify specific clones with much fewer individual assays.

**111.** (B) Faster separation and automated data collection.
Capillary electrophoresis revolutionized Sanger sequencing by replacing large, cumbersome slab gels. The use of narrow capillaries allows for the application of much higher voltages without excessive heat generation, resulting in significantly faster separation of DNA fragments. Furthermore, the system is fully automated, allowing for continuous sample loading and real-time detection of the fluorescently labeled fragments as they pass the detector.

**112.** (A) II, III and V
SMRT sequencing is a single-molecule technique that utilizes zero-mode waveguides to monitor the incorporation of fluorescently labeled nucleotides by a DNA polymerase in real-time. This method is noted for generating very long reads, often exceeding ten kilobases. It is incorrect to say it requires template amplification, as it works on single molecules, and it does not use a wash-cycle-based approach.

**113.** (A) Immortal, HGPRT-deficient, and do not secrete endogenous antibodies.
For successful hybridoma technology, the myeloma partner must possess specific traits. It must be an immortal cell line that can grow indefinitely in culture. Crucially, it must be deficient in the enzyme HGPRT to allow for HAT selection. Finally, it should not produce its own endogenous antibodies, ensuring that the resulting hybridoma only secretes the specific monoclonal antibody derived from the B-cell partner.

**114.** (C) Low absorbance signal
In a competitive ELISA, the target antigen in the sample competes with a fixed amount of labeled antigen for a limited number of antibody binding sites. When the concentration of the sample antigen is high, it successfully occupies most of the sites, leaving very few for the labeled antigen. Since the signal is generated by the labeled version, a high antigen concentration results in a low signal.

**115.** (A) Tyrosine
Radioimmunoassay requires the use of a radiolabeled version of the target antigen. For proteins and peptides, labeling is most commonly achieved by incorporating the radioactive isotope Iodine-125. This isotope is typically attached to the phenolic ring of tyrosine residues through a chemical or enzymatic iodination process. The presence of this radioactive tag allows for the precise measurement of the antigen during the assay.

**116.** (D) Fluorescence In Situ Hybridization (FISH)
While techniques like Southern blotting can identify the presence of a gene in a sample, they cannot determine its physical location on a chromosome. FISH provides the highest resolution for this purpose by allowing a fluorescently labeled probe to hybridize directly to a specific sequence on a metaphase chromosome spread. This enables researchers to visually pinpoint the gene to a specific chromosomal band.

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**117.** (B) It stops migrating and forms a discrete band (isopycnic point).
Isopycnic, or equilibrium, centrifugation separates particles based purely on their density. As the particles are subjected to centrifugal force, they move through the gradient until the density of the medium equals their own buoyant density. At this point, the net force on the particle becomes zero, and it stops moving, forming a highly concentrated and discrete band at its equilibrium position.

**118.** (C) A 100 kDa globular protein
In gel filtration chromatography, molecules are separated by their ability to enter the pores of the matrix beads. Larger molecules that are bigger than the pore size are completely

**189.** (D) Elute in the void volume
In gel filtration chromatography, the matrix beads contain pores of a specific size. Molecules that are larger than these pores are physically unable to enter the interior of the beads. These large molecules are restricted to the liquid space surrounding the beads. As a result, they travel through the column much faster than smaller molecules that get diverted into the pores, and they elute first in the void volume.

**190.** (A) Equilibrate column in high salt → Apply total RNA in high salt → Wash in high salt → Elute mRNA in low salt/water
The binding of the poly-A tail of mRNA to an oligo-dT column requires high salt concentrations to neutralize the negative charges and allow for stable base pairing. Therefore, the column is equilibrated and the sample is applied in a high-salt buffer. After washing away non-polyadenylated RNA with more high-salt buffer, the mRNA is released by switching to a low-salt buffer or pure water, which destabilizes the hydrogen bonds.

**191.** (B) It is highly active in the presence of SDS and EDTA.
Proteinase K is a highly robust and non-specific serine protease that is essential for genomic DNA extraction. Its unique advantage is its stability and high level of activity under harsh conditions that would inactivate most other enzymes. It remains fully functional in the presence of detergents like SDS, which denature other proteins, and chelating agents like EDTA, making it ideal for digesting proteins during the initial cell lysis step.

**192.** (D) Both genomic and plasmid DNA denature into single strands.
At the very high pH reached during the addition of sodium hydroxide (Solution II), the hydrogen bonds that hold DNA strands together are disrupted. This causes both the large bacterial genomic DNA and the small plasmid DNA to denature into single strands. While the genomic DNA strands separate completely, the circular strands of the plasmid DNA remain physically interlinked, which is the key to their subsequent separation during neutralization.

**193.** (A) Deactivate RNases and physically disrupt cell wall.
Extracting high-quality RNA from plant tissues is difficult due to the presence of a rigid cell wall and high levels of secondary metabolites and RNases. Flash-freezing the tissue in liquid nitrogen makes it brittle, allowing it to be ground into a fine powder, which physically breaks the cell walls. Furthermore, keeping the sample at extremely low temperatures throughout the process effectively prevents any RNase activity until the denaturing lysis buffer is added.

**194.** (C) Fluorometry using PicoGreen dye
Standard UV spectrophotometry is limited by its sensitivity and the fact that it detects all absorbing substances at 260 nanometers. For very dilute samples, fluorometric methods are significantly better. Dyes like PicoGreen are highly specific for double-stranded DNA and produce a fluorescent signal that is proportional to the concentration. This method can detect DNA at concentrations that are far below the detection limit of a typical spectrophotometer.

**195.** (A) Gel fails to conduct current, DNA will not migrate.
Gel electrophoresis requires an electrolyte solution to conduct the electric current from the electrodes through the gel. Standard buffers like TAE or TBE provide the necessary ions for this conductivity. Pure water has very few ions and is a very poor conductor of electricity. If water is used instead of a buffer, the current will be extremely low, and the DNA molecules will not experience the force required to move through the matrix.

**196.** (B) III, II, I
Each cycle of a polymerase chain reaction consists of three distinct temperature steps. First is denaturation, usually around ninety-five degrees, to separate the DNA strands. Next is annealing, where the temperature is lowered to allow primers to bind to their target sites. Finally, the temperature is raised to seventy-two degrees for extension, where the DNA polymerase synthesized the new strands. This cycle is repeated many times for amplification.

**197.** (B) Polymerase Chain Reaction (PCR)
Southern blotting was the original method for detecting the presence and size of specific DNA sequences within a genome. However, it is a

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they do not significantly retard the movement of the proteins. This allows the proteins to migrate quickly and accumulate into a very thin, sharp band at the interface, ensuring high-resolution separation based on size.

**128.** (D) Single-stranded and complementary to the target sequence
For a probe to successfully identify its target sequence on a Southern blot membrane, it must be able to form a stable double-stranded duplex. This is only possible if the probe itself is single-stranded and possesses a nucleotide sequence that is complementary to the target. During the hybridization process, the probe will find and bind to the fixed target through specific hydrogen bonding.

**129.** (A) Its expression level and transcript size
Northern blotting is a fundamental technique used to study gene expression at the RNA level. By separating total RNA or mRNA by size and then probing for a specific transcript, researchers can determine two key pieces of information. First, the intensity of the signal provides a measure of the gene's expression level. Second, the position of the band reveals the precise size of the transcript.

**130.** (B) II only
Random hexamers are short, six-nucleotide primers that bind randomly across all RNA molecules, allowing for cDNA synthesis from all species, including rRNA and mRNA. This is the only correct statement. MMLV typically has a lower optimal temperature than AMV. Oligo-dT primers bind to the poly-A tail at the three-prime end of the mRNA, not the five-prime cap structure.

**131.** (A) The background fluorescence level during the initial cycles of PCR.
In a real-time PCR amplification plot, the "baseline" refers to the signal detected during the early cycles of the reaction, typically cycles three through fifteen. During this phase, there is no significant increase in fluorescence above the background level because the amount of product is still very low. Correcting for this baseline is essential for the accurate determination of the cycle threshold value later.

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**132.** (D) A higher rate of nucleotide misincorporation
Proofreading activity, which is the three-prime to five-prime exonuclease function of certain DNA polymerases, allows the enzyme to remove incorrectly incorporated nucleotides. Taq polymerase naturally lacks this proofreading ability. Consequently, it has a higher error rate compared to proofreading enzymes like Pfu. This results in a higher frequency of point mutations in the final amplified PCR products, which can be problematic for cloning.

**133.** (B) The number of plaques or colonies per volume of plated dilution
The titer of a genomic or cDNA library represents the concentration of infectious particles or recombinant clones present in the library stock. It is determined by performing a serial dilution of the library and plating it on a suitable host. The number of resulting plaques for phage libraries or colonies for plasmid libraries is then counted and used to calculate the total number of clones.

**134.** (C) Prevent enzyme from cutting internal sites in cDNA.
Linkers containing restriction sites are ligated to the ends of cDNA to facilitate cloning. After ligation, these linkers must be digested with the corresponding restriction enzyme to create sticky ends. However, the same restriction site might also exist internally within the cDNA sequence. To prevent the enzyme from cutting and destroying the target cDNA, the internal sites are typically protected by treatment with a methyltransferase.

**135.** (D) Applying secondary antibody followed by substrate.
In the immunological screening of an expression library, the bacterial colonies are lysed to release the expressed proteins. A primary antibody is then added to bind specifically to the protein of interest. To visualize this binding, a secondary antibody conjugated to an enzyme is applied. When the appropriate chromogenic or chemiluminescent substrate is added, it produces a signal that identifies the location of the positive clone.

**136.** (A) Lack of 3'-OH prevents phosphodiester bond formation.
During DNA synthesis, the polymerase adds a new nucleotide by creating a phosphodiester bond between the five-prime phosphate of the

excluded and travel only in the space between the beads. Consequently, they take the shortest possible path and elute first from the column. Therefore, a one hundred kilodalton protein will elute before smaller proteins or salts.

**119.** (D) Bonded by three hydrogen bonds, requiring more energy.
The stability of a DNA double helix is determined by the hydrogen bonding between the complementary base pairs. Guanine-cytosine pairs are held together by three hydrogen bonds, whereas adenine-thymine pairs are held together by only two. Because of this extra bond, G-C pairs require significantly more thermal energy to be broken. Therefore, DNA fragments with a higher GC content exhibit a higher melting temperature.

**120.** (A) P-2, Q-1, R-4, S-3
Ethidium bromide is an intercalating agent that slides between DNA base pairs. SDS is an anionic detergent that binds to proteins, giving them a uniform negative charge. Acrylamide is the chemical monomer that polymerizes to create the porous matrix for protein separation. Tracking dyes are small molecules that migrate quickly with the solvent front, providing a visual marker for the progress of the run.

**121.** (B) Binds DNA reversibly in high chaotropic salt concentrations.
The silica membrane within a spin column provides a solid phase for the selective capture of nucleic acids. Under conditions of high chaotropic salt concentration and appropriate pH, DNA molecules bind to the silica surface. This allows contaminants to be washed away while the DNA remains immobilized. The purified DNA is then subsequently eluted from the membrane using a low-salt buffer or pure water.

**122.** (B) The successful precipitation of SDS, proteins, and genomic DNA.
In the alkaline lysis protocol, the addition of the acidic neutralization buffer (Solution III) is a critical step. The potassium acetate rapidly lowers the pH, which causes the large genomic DNA and cellular proteins to precipitate. Furthermore, the potassium ions react with the SDS to form an insoluble salt. The visible cloudiness indicates that these components have successfully aggregated and can now be removed by centrifugation.

**123.** (A) Extracts lipids and stabilizes phase separation.
Chloroform is a dense organic solvent used in conjunction with phenol during nucleic acid extraction. Its primary function is to facilitate the clear and sharp separation of the aqueous phase from the organic phase. Additionally, chloroform helps to denature proteins and effectively removes lipids and traces of phenol from the aqueous phase, ensuring the purity of the recovered RNA in the upper layer.

**124.** (C) Proteins
A pure RNA sample typically has an $\frac{A_{260}}{A_{280}}$ ratio of approximately 2.0. Proteins have a maximum absorbance at 280 nanometers because of their aromatic amino acids. If the ratio is significantly low, such as 1.4, it indicates that the sample is heavily contaminated with protein impurities. This usually happens when the protein removal steps during the extraction process, like phenol treatment, were not completely successful.

**125.** (D) OliGreen
Different fluorescent dyes have been developed for the specific quantification of different types of nucleic acids. PicoGreen is highly specific for double-stranded DNA, while RiboGreen is used for RNA. For the quantification of single-stranded DNA, such as synthetic oligonucleotides or denatured genomic DNA, OliGreen is the most appropriate and sensitive choice, as it exhibits a massive increase in fluorescence upon binding to single strands.

**126.** (A) Restriction digestion → Gel electrophoresis → Denaturation → Membrane transfer → Hybridization
A Southern blot begins by digesting genomic DNA with restriction enzymes. The resulting fragments are separated by size using gel electrophoresis. Because the DNA is double-stranded, it must be denatured within the gel before being transferred to a membrane. Once fixed to the membrane, the target sequence is identified by incubating it with a labeled probe that hybridizes to its complement.

**127.** (B) Allow proteins to migrate rapidly and concentrate at the interface.
The stacking gel in SDS-PAGE is designed to concentrate the sample before it enters the resolving gel. By using a very low percentage of acrylamide, the pores are large enough that

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**145.** (A) It solubilizes the lipid bilayer of the cell membrane.
Sodium dodecyl sulfate is a powerful anionic detergent used in many lysis buffers. It works by disrupting the structural integrity of the cell. The hydrophobic tails of the SDS molecules insert themselves into the lipid bilayer of the cell membrane and the nuclear envelope. This action solubilizes the lipids and denatures the membrane proteins, effectively causing the cell to break open and release its contents.

**146.** (C) Powerful chaotropic agent that denatures endogenous RNases.
RNA is extremely sensitive to degradation by ribonucleases, which are present everywhere. Guanidinium thiocyanate is one of the most effective reagents for RNA extraction because it is a very strong chaotropic agent. It rapidly denatures all cellular proteins, including the highly stable and active RNases. By instantly inactivating these enzymes at the moment of cell lysis, it ensures that the RNA remains intact and high-quality.

**147.** (C) 40 µg/mL
In UV spectrophotometry, the concentration of a nucleic acid sample is calculated using a standard conversion factor. For pure RNA, an absorbance of 1.0 at a wavelength of 260 nanometers, using a one-centimeter path length, corresponds to a concentration of approximately forty micrograms per milliliter. This is slightly lower than the fifty micrograms per milliliter used for double-stranded DNA due to the different extinction coefficients.

**148.** (D) Hydrogen bonding and entanglements of polysaccharide chains.
Agarose is a linear polysaccharide derived from seaweed. When agarose is dissolved in boiling buffer and then cooled, it does not undergo a chemical reaction. Instead, the individual polymer chains form helical structures that aggregate into a three-dimensional network. This gelation is driven by physical interactions, primarily hydrogen bonding and the physical entanglement of the chains, creating a porous matrix suitable for electrophoresis.

**149.** (B) SDS and reducing agents
In standard SDS-PAGE, proteins are completely denatured and their subunits are separated because of the detergent and reducing agents like β-mercaptoethanol. Native PAGE omits these harsh chemicals, allowing proteins to remain in their original, folded state. Because the tertiary structure and subunit associations are preserved, the proteins often maintain their biological functions, such as enzymatic activity or the ability to bind specific ligands.

**150.** (C) A is true but R is false.
In a traditional Southern blot, the transfer of DNA from the gel to the membrane is not driven by electricity. Instead, it relies on capillary action. A reservoir of buffer is drawn upward through the gel and the membrane by the absorbent paper towels on top. As the buffer moves, it carries the DNA fragments along with it, where they become trapped and fixed on the surface of the membrane.

**151.** (D) Fluorescently labeled specific antibody
Northern blotting is designed to detect and quantify specific RNA sequences. This is achieved using probes that are complementary to the target RNA, such as labeled DNA, cRNA, or oligonucleotides. Antibodies are proteins that bind to other proteins or specific antigens, not to specific nucleotide sequences. Therefore, an antibody would be completely unsuitable as a probe for identifying an RNA transcript on a Northern blot membrane.

**152.** (A) Binding to all empty, unoccupied hydrophobic sites on membrane.
Blocking is a crucial step in Western blotting that follows the transfer of proteins to the membrane. Milk contains a high concentration of casein and other proteins that have a natural affinity for the membrane. By soaking the membrane in a milk solution, these proteins bind to all the areas where the target proteins are not present, preventing the antibodies from sticking non-specifically to the membrane surface.

**153.** (B) DMSO (Dimethyl sulfoxide)
DNA sequences with a high percentage of guanine and cytosine are often difficult to amplify because they form very stable double helices and secondary structures that resist denaturation. Dimethyl sulfoxide is a common additive used to solve this problem. It works by destabilizing the DNA duplex, effectively lowering the melting temperature. This makes it easier for the polymerase to navigate and synthesize across these challenging, GC-rich regions.

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**154.** (A) Both A and R are true and R is the correct explanation of A.
End-point PCR only measures the final amount of product after the reaction is finished, which is often in the plateau phase where reagents are exhausted. Real-time PCR is superior for quantification because it monitors the amplification as it occurs. By measuring the signal during the exponential phase, where the reaction efficiency is constant and maximal, researchers can accurately calculate the initial starting concentration of the target template.

**155.** (A) A cDNA library intended for immunological screening
Immunological screening is a method used to find specific clones by detecting the protein they produce. For this to work, the library must be constructed using an expression vector. This type of vector contains all the necessary bacterial regulatory elements, such as a strong promoter and a ribosome binding site, to ensure that the cloned eukaryotic cDNA is transcribed and then translated into a protein by the host.

**156.** (C) The assembly of recombinant lambda DNA into mature infectious phage particles.
In vitro packaging is a laboratory technique used to create infectious bacteriophage particles. Recombinant lambda DNA is mixed with purified phage head and tail proteins in a test tube. These components spontaneously assemble around the DNA, creating a mature virus. This process is essential for building large genomic libraries because the resulting phages can infect bacterial cells much more efficiently than naked DNA can be transformed.

**157.** (D) Plaque hybridization
Plaque hybridization is a technique used to screen a bacteriophage library for a specific gene. It relies on the principle of nucleic acid hybridization. A labeled probe, which consists of a DNA or RNA sequence that is complementary to the target gene, is incubated with a membrane containing DNA from the phage plaques. The probe will bind only to the plaques that contain the matching sequence, allowing for their identification.

**158.** (C) Capillary electrophoresis fails to resolve single-base diff.
In Sanger sequencing, the DNA fragments are separated by size to determine the sequence. As the fragments get longer, the relative difference in size between a fragment of, say, one thousand bases and one thousand and one bases becomes extremely small. Eventually, the resolution of the capillary electrophoresis system is no longer sufficient to distinguish between these single-base differences, which sets the practical limit for a single sequencing read.

**159.** (B) Generate dense localized clusters of identical molecules.
Bridge amplification is a unique process used in Illumina sequencing to prepare the DNA template on a flow cell. Each single DNA molecule is amplified in place, forming a tiny "bridge" between immobilized primers on the surface. This results in the creation of a dense cluster containing thousands of identical copies of the original molecule. These clusters are necessary because a single molecule would not produce enough light to be detected.

**160.** (A) I, II and IV
Monoclonal antibodies are identical molecules produced by a single hybridoma clone, and they bind to only one specific epitope. The process requires HAT medium to select for the successfully fused cells. However, it is incorrect to say they are harvested from serum or have high variability; those are characteristics of polyclonal antibodies. Monoclonal antibodies are known for their high specificity and consistency across different production batches.

**161.** (A) Multiple secondary antibodies bind a single primary antibody.
The primary advantage of an indirect ELISA is the amplification of the signal. Because multiple enzyme-labeled secondary antibodies can bind to different epitopes on the constant region of a single primary antibody, the resulting signal is much stronger than in a direct ELISA. This increased sensitivity allows for the detection of lower concentrations of the target antigen in the sample, improving the overall performance of the assay.

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**162.** (C) Low amount of radioactivity in the bound complex
In a competitive radioimmunoassay for thyroid hormones, the unlabeled hormone from the patient's sample competes with a fixed amount of radiolabeled hormone for antibody binding sites. If the patient has an abnormally

high concentration of the hormone, it will displace most of the radioactive tracer from the antibodies. Consequently, when the bound complex is measured, it will show a very low level of radioactivity.

**163.** (A) Lower $T_m$, allowing hybridization at lower temperatures.
In fluorescence in situ hybridization, the DNA must be kept in a single-stranded state for the probe to bind. Formamide is a chemical denaturant that weakens the hydrogen bonds between DNA strands, effectively lowering the melting temperature of the duplex. This allows the hybridization process to occur at a lower, more gentle temperature, which helps to preserve the delicate morphology of the chromosomes during the assay.

**164.** (D) The volume of the mobile phase located outside the porous beads.
In gel filtration chromatography, the total volume of the column is composed of the volume within the porous beads and the volume surrounding them. The "void volume" specifically refers to the space outside of the beads. Any molecule that is too large to enter the pores of the beads will travel only through this external space and will elute from the column as soon as this volume has passed.

**165.** (B) Competitively displaces histidine from nickel on the matrix.
Purification of His-tagged proteins relies on the affinity between histidine residues and nickel ions. Imidazole is the side-chain of the amino acid histidine. By adding a high concentration of free imidazole to the elution buffer, it acts as a competitor. The imidazole molecules outcompete the His-tagged protein for the binding sites on the nickel-NTA matrix, causing the protein to be released and eluted from the column.

**166.** (C) Likely contains significant RNA contamination.
While the $\frac{A_{260}}{A_{280}}$ ratio for pure DNA is between 1.8 and 2.0, RNA naturally has a higher ratio of approximately 2.0 or slightly above. This is because uracil and other features of RNA contribute to a higher absorbance at 260 nanometers relative to 280. If a DNA sample shows a ratio of 2.1, it is an indication that the sample likely contains residual RNA contamination.

**167.** (D) A monovalent cation (e.g., Sodium or Ammonium).
For DNA to precipitate from an aqueous solution upon the addition of ethanol, the negative charges on its phosphate backbone must be neutralized. This requires the presence of a high concentration of monovalent cations, such as sodium or ammonium ions. These cations form ionic bonds with the phosphate groups, reducing the DNA's solubility and allowing it to aggregate and form a solid pellet during centrifugation.

**168.** (A) P-3, Q-4, R-1, S-2
Solution I contains EDTA to chelate divalent cations and glucose for osmotic stability. Solution II uses NaOH and SDS to lyse the cells and denature the DNA. Solution III contains potassium acetate to neutralize the pH, causing genomic DNA and proteins to precipitate. Finally, isopropanol is used to concentrate and precipitate the purified plasmid DNA from the resulting supernatant.

**169.** (B) Poly-A tail exclusively on eukaryotic mRNA.
Eukaryotic cells contain various types of RNA, with ribosomal RNA being the most abundant. However, only messenger RNA molecules possess a long string of adenine residues at their three-prime end, known as the poly-A tail. Oligo-dT chromatography uses a matrix with attached thymidine chains that specifically hybridize to this tail. This allows mRNA to be selectively captured while rRNA and other non-polyadenylated species are washed away.

**170.** (B) $A = \log\left(\frac{1}{T}\right)$
Absorbance and transmittance are two related ways of measuring how much light is absorbed by a sample in a spectrophotometer. Transmittance is the fraction of light that passes through the sample. Absorbance is defined as the base-ten logarithm of the reciprocal of the transmittance. This logarithmic relationship is useful because absorbance is directly proportional to the concentration of the substance, according to the Beer-Lambert law.

**171.** (A) Intercalates base pairs, causing frameshift mutations.
Ethidium bromide is a flat, planar molecule that has a similar shape to the nitrogenous base pairs in DNA. This allows it to slide, or intercalate, between the stacked bases of the double helix. During DNA replication or transcription, this

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incoming nucleotide and the three-prime hydroxyl group of the existing chain. Dideoxynucleotides are engineered to lack the three-prime hydroxyl group. Once a dideoxynucleotide is incorporated into the strand, the polymerase cannot add any more nucleotides, leading to the termination of that specific DNA fragment.

**137.** (B) Detects light emission vs. size separation.
Traditional Sanger sequencing relies on the production of chain-terminated fragments of varying lengths, which are then separated by size using electrophoresis. In contrast, pyrosequencing is a sequencing-by-synthesis method that monitors the reaction in real-time. It detects the release of pyrophosphate each time a nucleotide is added, which is then converted into a light signal. This eliminates the need for any gel electrophoresis.

**138.** (B) Intrinsically mortal and cannot survive long-term in vitro.
HAT medium is designed to select for hybridoma cells by killing unfused myeloma cells, which lack the HGPRT enzyme. While normal B-cells do possess the salvage pathway enzyme and can theoretically survive the aminopterin blockade, they are primary cells with a very limited lifespan. In a typical culture environment, unfused B-cells will naturally die within a few days because they are not immortalized like hybridomas.

**139.** (A) Non-specific binding of labeled primary antibody to plate.
In a direct ELISA, the primary antibody is directly labeled with an enzyme. The main challenge with this format is that the labeled antibody itself may bind non-specifically to the plastic surface of the microtiter plate or to other non-target proteins. Since every bound antibody produces a signal, this non-specific binding leads to high background noise, which can reduce the overall sensitivity and accuracy of the assay.

**140.** (B) II only
FISH is indeed powerful for detecting microdeletions and can be performed on interphase cells without needing them to divide. However, the statement that FISH probes are typically labeled with radioactive isotopes is incorrect. As the name implies, Fluorescence In Situ Hybridization utilizes fluorescent dyes to label the DNA probes. This allows for safe, high-resolution visualization and the ability to use multiple colors in a single sample.

**141.** (D) Frictional coefficient (shape)
The sedimentation coefficient of a protein or particle during ultracentrifugation is a measure of how fast it moves through a solvent. This value is directly proportional to the particle's mass; heavier particles sediment faster. However, it is also inversely proportional to the frictional coefficient, which is determined by the particle's shape and size. A more extended or less compact shape increases friction and slows down sedimentation.

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**142.** (C) Positively charged to bind negatively charged molecules
Ion-exchange chromatography separates molecules based on their net surface charge. Anion-exchange chromatography specifically uses a stationary phase that is positively charged, such as diethylaminoethyl (DEAE) groups. This positive matrix attracts and binds negatively charged molecules, or anions. Proteins with a net negative charge at the operating pH will bind to the column, while positively charged or neutral proteins will pass through.

**143.** (B) The sequence length and its GC content
The melting temperature of a DNA duplex is the temperature at which half of the molecules are denatured into single strands. This value is primarily determined by the nucleotide sequence. Longer sequences have more total hydrogen bonds and thus higher melting points. Additionally, because guanine-cytosine pairs are held by three hydrogen bonds compared to two in adenine-thymine pairs, a higher GC percentage significantly increases the stability.

**144.** (D) RNA will partition into the aqueous phase, while DNA partitions into the organic/interphase.
The partitioning of nucleic acids during phenol extraction is highly dependent on the pH of the organic solvent. At an acidic pH of 4.5, the phosphate groups on the DNA backbone become neutralized. This makes the DNA much less polar, causing it to partition into the organic phase or the interphase. RNA remains polar enough to stay in the aqueous phase, allowing for its selective purification from DNA.

reverse transcriptase. Terminal transferase is another enzyme often used in cDNA cloning to add homopolymeric tails, such as poly-C, to the ends of the cDNA to facilitate ligation. Statement II is incorrect because cDNA libraries are constructed from a messenger RNA template, not from genomic DNA.

**181.** (B) A specific antibody and the protein expressed by the clone
Immunological screening is a method used to identify a specific recombinant clone within an expression library. Unlike hybridization methods that look for a DNA sequence, this technique identifies the target based on the presence of its encoded protein product. Bacterial colonies are lysed, and the released proteins are bound to a membrane. A specific antibody is then used to find the clone that is producing the target protein.

**182.** (A) II only
Sanger sequencing correctly uses a polymerase to extend a primer, and capillary electrophoresis resolves fragments by size. Chromatogram peaks are indeed color-coded by base. However, the statement that dideoxynucleotides possess a three-prime hydroxyl group is incorrect. The defining feature of ddNTPs is the absence of this three-prime hydroxyl group, which is what causes the termination of the DNA strand once they are incorporated.

**183.** (D) It performs sequencing on single unamplified DNA molecules.
One of the most significant differences between PacBio SMRT sequencing and Illumina is the treatment of the DNA template. Illumina requires a prior amplification step to create clusters of identical molecules to produce a detectable signal. In contrast, SMRT sequencing is a single-molecule technique. It observes the activity of a single DNA polymerase enzyme as it processes a single, unamplified DNA template, allowing for the detection of base modifications.

**184.** (C) 1 in $10^5$ cells
The fusion of somatic cells, such as B-cells and myeloma cells, is an extremely rare and inefficient event. Even with the use of chemical fusogens like polyethylene glycol or electrical pulses, only a very small fraction of the total cells will successfully fuse to form a viable hybridoma. In a typical fusion experiment, the successful frequency is approximately one hybrid cell for every one hundred thousand starting cells.

**185.** (B) A capture antibody specific to the target antigen.
The first step in a sandwich ELISA is to immobilize a specific “capture” antibody onto the surface of the microtiter plate wells. This antibody is chosen for its ability to bind the target antigen with high affinity and specificity. When the sample is added later, the capture antibody effectively “fishes” the target antigen out of the complex mixture and holds it in place for subsequent detection.

**186.** (B) Ratio of bound to free vs. unlabeled antigen.
A standard curve in radioimmunoassay is essential for determining the concentration of the antigen in unknown samples. It is created by performing the assay with a range of known concentrations of the unlabeled antigen. The results are typically plotted as the ratio of antibody-bound radioactivity to free radioactivity, or the percentage of bound tracer, against the concentration of the unlabeled antigen on a logarithmic scale.

**187.** (A) The degree of chromatin condensation
The spatial resolution of a FISH assay, which refers to the ability to distinguish between two close signals, is heavily influenced by the state of the DNA. In metaphase chromosomes, the DNA is highly condensed, which allows for the mapping of sequences to specific bands but limits the resolution to several megabases. Using less condensed interphase nuclei or stretched DNA fibers significantly increases the resolution, allowing for finer mapping.

**188.** (C) Successively increasing centrifugal forces and times
Differential centrifugation is a step-by-step process used to separate cellular organelles by their size and density. The process begins with a low-speed spin to pellet the largest and densest components, like whole cells and nuclei. The resulting supernatant is then transferred to a new tube and spun at a higher speed to pellet smaller organelles like mitochondria. This cycle is repeated at increasingly higher speeds to isolate smaller components.

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distortion of the DNA structure can cause the polymerase to add or skip bases, leading to frameshift mutations, which is why it is handled with extreme care.

**172.** (C) Reduce intra- and inter-molecular disulfide bonds.
Many proteins are composed of multiple subunits or are held in a specific folded shape by covalent disulfide bonds between cysteine residues. To ensure that proteins are separated purely by the length of their polypeptide chains in SDS-PAGE, these bonds must be broken. B-mercaptoethanol or dithiothreitol are reducing agents added to the sample buffer to break these disulfide bridges, resulting in completely linear, individual polypeptides.

**173.** (D) Breaks DNA into smaller fragments to facilitate transfer.
In Southern blotting, large genomic DNA fragments can become trapped within the tight pores of the agarose gel, making their transfer to the membrane very slow and inefficient. Treating the gel with a dilute acid like hydrochloric acid removes some of the purine bases. This weakens the DNA backbone, causing it to break into smaller pieces that can more easily migrate out of the gel and onto the membrane.

**174.** (C) RNA is highly susceptible to ubiquitous and stable RNases.
The primary difficulty in working with RNA, as compared to DNA, is its extreme fragility and the ubiquity of ribonucleases. RNases are exceptionally stable enzymes that do not require metal cofactors and can survive standard autoclaving. They are present on human skin and in most laboratory environments. Therefore, maintaining an RNase-free environment is the most critical and challenging aspect of performing a successful Northern blot.

**175.** (B) An electrophoretic current (electroblotting)
After proteins have been separated by SDS-PAGE, they must be moved from the gel onto a membrane for detection. Because the proteins are coated with SDS, they carry a negative charge. In the electroblotting process, the gel and membrane are placed in a sandwich and subjected to an electric field. This field drives the negatively charged proteins out of the gel and onto the positively charged side.

**176.** (D) Annealing temperature and magnesium ion concentration.
The specificity of a PCR reaction depends on the stringency with which the primers bind to the template. Two factors are most commonly adjusted to optimize this. Increasing the annealing temperature makes the binding more selective for perfectly matched sequences. Additionally, magnesium ions act as a cofactor for the polymerase and stabilize DNA duplexes; adjusting their concentration can significantly affect the balance between yield and specificity.

**177.** (A) Process occurs in a single tube without opening it.
In a one-step RT-PCR system, all the necessary components for both reverse transcription and PCR amplification are added to a single reaction tube at the beginning. The thermal cycler is then programmed to perform the reverse transcription at a lower temperature, followed immediately by the high-temperature PCR cycles. This approach minimizes handling and the risk of cross-contamination, though it offers less flexibility than two-step protocols.

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**178.** (C) Fluorescence Resonance Energy Transfer (FRET)
TaqMan probes are designed with a fluorescent reporter dye at one end and a quencher molecule at the other. When the probe is intact, the quencher is close enough to the reporter to absorb its signal via fluorescence resonance energy transfer. During the extension phase, the five-prime nuclease activity of the DNA polymerase cleaves the probe, separating the dye from the quencher and allowing the fluorescence to be detected.

**179.** (A) Enzyme concentration and incubation time
To achieve a partial restriction digest, the reaction conditions are intentionally limited so that the enzyme does not cut at every possible recognition site in the DNA sample. This is typically accomplished by using a very small amount of the restriction enzyme or by significantly shortening the total incubation time. This results in a population of overlapping DNA fragments, which is essential for constructing a representative genomic library.

**180.** (B) I and III
The synthesis of the first DNA strand from an RNA template is catalyzed by the enzyme

labor-intensive and time-consuming process that requires large amounts of DNA. It has been largely replaced by PCR and its variants, which are much faster, more sensitive, and can work with minute quantities of DNA. PCR can quickly amplify a specific target sequence, making it easy to detect.

**198.** (C) It does not require a fume hood and is less toxic.
Formaldehyde is the most traditional denaturant used in Northern blotting, but it is a hazardous, volatile chemical that must be used inside a fume hood to prevent inhalation of toxic vapors. Glyoxal is a non-volatile alternative that provides excellent denaturation of RNA by forming stable adducts with the bases. Because it is much less hazardous to handle and does not produce toxic fumes, it is preferred in many laboratory settings.

**199.** (D) Bovine serum albumin (BSA) or non-fat dried milk.
Blocking is a critical step in Western blotting that occurs after the proteins are transferred to the membrane. To prevent the antibodies from sticking non-specifically to the empty spaces on the membrane, it is incubated with a solution containing an excess of inert proteins. Solutions of bovine serum albumin or non-fat dried milk are the most common blocking agents, as they effectively coat the membrane and reduce background noise.

**200.** (A) I only
In qPCR, the lower the Ct value, the more template was present initially, and probes like TaqMan or dyes like SYBR Green are standard detection tools. However, the statement about the threshold line is incorrect. The threshold line must be set during the exponential phase of the amplification curve, where the increase in signal is most consistent. Setting it in the plateau phase would be useless for accurate quantification.

**201.** (B) RNase H
In the process of reverse transcription, an RNA-DNA hybrid is formed. For the subsequent PCR step to be most efficient, the original RNA template should be removed. RNase H is a specific enzyme that recognizes and degrades the RNA strand within an RNA-DNA duplex. By adding RNase H after the first-strand synthesis, the cDNA is freed from the RNA, making it more accessible for the DNA polymerase during the PCR cycles.

**202.** (B) Logarithmic fluorescence vs. linear cycle number
A standard real-time PCR amplification plot displays the accumulation of the fluorescent signal over the course of the reaction. Because the DNA is amplified exponentially, the signal also increases exponentially. To make this data easier to analyze and to identify the linear exponential phase, the fluorescence intensity is typically plotted on a logarithmic scale (y-axis) against the cycle number on a linear scale (x-axis).

**203.** (A) Bacterial Artificial Chromosome (BAC).
Different cloning vectors have different capacities for the size of the DNA insert they can carry. Plasmids and lambda phages are limited to relatively small fragments. For studying large eukaryotic genomes, vectors like Bacterial Artificial Chromosomes are preferred. BACs are based on the F-plasmid of bacteria and can stably maintain and replicate very large inserts, often ranging from one hundred to three hundred kilobases in size.

**204.** (C) 1-5%
When total RNA is extracted from a cell, it is composed of several different types of RNA molecules. The vast majority, roughly eighty to eighty-five percent, is ribosomal RNA, while transfer RNA makes up most of the remainder. Messenger RNA, despite being the target for cDNA library construction, is a very small fraction of the total pool, typically representing only one to five percent of the total cellular RNA.

**205.** (D) Recombinant plasmids where lacZ' is disrupted by insert.
Blue-white screening is used to identify bacterial colonies that contain a recombinant plasmid. The vector carries a gene called lacZ', which produces an enzyme that turns a substrate blue. When a foreign DNA fragment is successfully inserted into the vector, it breaks the lacZ' gene, preventing the production of the functional enzyme. Therefore, colonies containing the recombinant plasmid remain white, while those with the original vector turn blue.

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# CHAPTER 07
# Gene Cloning

2025

**1.** Which class of restriction endonucleases cleaves DNA at specific sites within or immediately adjacent to their recognition sequences and is most commonly used in recombinant DNA technology?
(A) Type I
(B) Type II
(C) Type III
(D) Type IV

2008

**2.** What term describes different restriction enzymes isolated from different organisms that recognize the exact same DNA sequence and leave the same cleavage pattern?
(A) Isoschizomers
(B) Isocaudomers
(C) Neoschizomers
(D) Homologous enzymes

2015

**3.** Type I restriction endonucleases differ from Type II because Type I enzymes typically cleave DNA:
(A) Exactly at the palindromic axis of symmetry
(B) Immediately outside the specific recognition site
(C) At distant, non-specific random sites
(D) Strictly at hemi-methylated DNA sequences

2016

**4.** Restriction enzymes that recognize slightly different sequences but produce identical complementary sticky ends are classified as:
(A) Isoschizomers
(B) Neoschizomers
(C) Restriction isomers
(D) Isocaudomers

2017

**5.** Which statement correctly identifies a distinguishing feature of neoschizomers?
(A) They strictly require ATP for restriction activity
(B) They are exclusively isolated from filamentous phages
(C) They cleave the same recognition sites at different positions
(D) They only cleave modified single-stranded DNA

2006

**6.** Identify the INCORRECT statement regarding Type III restriction endonucleases.
(A) They require both ATP and SAM as essential cofactors
(B) They possess separate, independent R and M enzymes
(C) They cleave DNA at specific distances from recognition sites
(D) Their recognition sequences are typically non-palindromic

2021

**7.** Which of the following is an exception to the general characteristics of typical Type II restriction endonucleases?
(A) Absence of an ATP requirement for DNA cleavage
(B) Functional requirement for active homodimer formation
(C) Essential requirement for divalent $Mg^{2+}$ cations
(D) Single polypeptide R-M bifunctional activity

2009

**8.** Under non-optimal conditions such as high glycerol concentration or high enzyme-to-DNA ratios, restriction enzymes may exhibit relaxed specificity. This phenomenon is termed:
(A) Star activity
(B) Methylation interference
(C) Non-specific binding
(D) Isoschizomerism

2013

**9.** Type IV restriction endonucleases uniquely target and cleave which type of DNA?
(A) Hemimethylated DNA only
(B) Completely unmethylated DNA
(C) Modified or methylated DNA
(D) Single-stranded RNA-DNA hybrids

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biology. The matrix is modified with a ligand that only a specific protein can recognize and bind to. Common examples include the binding between an enzyme and its substrate, an antibody and its antigen, or a hormone and its receptor. This allows the target protein to be purified in a single step.

**215.** (D) NA absorb at 260, proteins at 280.
Spectrophotometric measurement of nucleic acid purity is based on the different light absorption properties of molecules. The nitrogenous bases in DNA and RNA absorb ultraviolet light most strongly at a wavelength of 260 nanometers. In contrast, the aromatic side chains of the amino acids tryptophan and tyrosine in proteins have a maximum absorption at 280 nanometers. By comparing the absorbance at these two points, the relative purity can be estimated.

**216.** (A) The pellet needs to be washed and dried quickly.
Both ethanol and isopropanol can be used to precipitate DNA, but ethanol is often preferred for the final steps. Ethanol is more volatile and has a lower boiling point, which means the resulting DNA pellet will dry much faster than one precipitated with isopropanol. Furthermore, ethanol is better at keeping salts in solution, which helps to produce a cleaner DNA pellet that is free of salt contamination.

**217.** (B) Plasmid DNA circularity keeps complementary strands linked.
During the denaturation phase of alkaline lysis, the two strands of the circular plasmid DNA become separated but remain physically intertwined like links in a chain. When the pH is neutralized, these strands are still in close proximity and can quickly and perfectly snap back together. The large genomic DNA strands, however, are linear and become completely separated, making it impossible for them to find their complements and renature correctly.

**218.** (A) Inactivate RNases by modifying amino acid residues.
Working with RNA requires strict precautions against ribonucleases. DEPC is a highly reactive chemical that is added to water and laboratory buffers to eliminate RNase activity. It works by covalently modifying the imidazole groups of histidine residues and other amino acids in the active site of the enzymes. After the treatment, the water is autoclaved to break down the DEPC into harmless carbon dioxide and ethanol.

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**219.** (A) 0.020 $(\mu g/mL)^{-1}$ $cm^{-1}$
The relationship between the concentration of a substance and its light absorbance is described by the extinction coefficient. For double-stranded DNA, it is generally accepted that a concentration of fifty micrograms per milliliter results in an absorbance of 1.0 at 260 nanometers. In the units of reciprocal micrograms per milliliter per centimeter, this corresponds to an extinction coefficient of exactly point zero two zero.

**220.** (A) P-2, Q-4, R-1, S-3
Gel filtration chromatography separates molecules based on their size and shape. Affinity chromatography relies on specific, reversible biological interactions like enzyme-substrate binding. Anion-exchange chromatography utilizes a positively charged matrix to bind negatively charged molecules. In contrast, cation-exchange chromatography uses a negatively charged matrix to capture and bind positively charged molecules, allowing for separation based on the net surface charge of the proteins.

**221.** (D) Its charge-to-mass ratio and its physical conformation
In Native PAGE, proteins are not denatured and do not have a uniform charge. Instead, they retain their original biological shape and internal charges. The speed at which a protein moves through the gel is determined by its net electrical charge and its size and shape, which together define its charge-to-mass ratio. Therefore, a small, highly charged protein will move much faster than a large, less charged, or irregularly shaped one.

**222.** (C) Electric field to drive proteins out of gel onto membrane.
After the separation of proteins by SDS-PAGE, they must be transferred to a stable membrane for further analysis. Electroblotting is the most common method for this transfer. It involves placing the gel in direct contact with a membrane and applying an electric current across the stack. This current drives the negatively charged proteins out of the gel matrix and onto the surface of the membrane, where they become immobilized.

highly pure and largely free of contaminating proteins or other aromatic impurities.

**240.** (D) I, II and III
Successful plasmid isolation requires several specific steps. RNase A must be included to remove the high volume of cellular RNA that would otherwise contaminate the sample. Solution II provides the SDS and sodium hydroxide necessary for lysis and denaturation. Finally, it is critical to use gentle inversion rather than vortexing during the lysis step to ensure that the large bacterial genomic DNA is not sheared into small fragments.

**241.** (B) It decreases the mobility by increasing the mass and reducing the net negative charge.
Ethidium bromide is a large molecule that intercalates between the base pairs of DNA. When it binds, it adds significant mass to the DNA molecule without adding any negative charge; in fact, the dye itself is positively charged, which partially neutralizes the DNA's negative backbone. Furthermore, the intercalation causes the DNA helix to unwind and lengthen. These combined factors increase the frictional resistance and decrease the overall electrophoretic mobility of the DNA.

**242.** (C) A coat protein displayed on the exterior surface.
Phage display is a powerful technique for studying protein interactions. It involves genetically engineering a bacteriophage so that the DNA sequence for a protein of interest is fused to the gene for one of the phage's own coat proteins. When the phage replicates, it "displays" the foreign protein on its exterior surface. This allows researchers to easily screen large libraries of proteins to find those that bind to a specific target molecule.

**243.** (A) Bands smeared and diffuse due to lack of pre-concentration.
The purpose of the stacking gel in SDS-PAGE is to concentrate the protein sample into a very thin, sharp starting zone before it enters the resolving gel. If this step is omitted and the sample is loaded directly onto the resolving gel, the proteins will enter the matrix at different times and positions over the entire volume of the well. This results in wide, smeared, and poorly resolved bands that are difficult to analyze.

**244.** (B) The temperature and salt concentration of the wash buffer.
After the hybridization step in a Southern blot, the membrane must be washed to remove any probe that has bound non-specifically or to partially matched sequences. The stringency of these washes is the most important factor for a clean result. Higher temperatures and lower salt concentrations increase the stringency, ensuring that only the most perfectly matched, stable DNA duplexes remain on the membrane while everything else is washed away.

**245.** (B) II only
PCR primers should generally have a GC content of forty to sixty percent and be between eighteen and twenty-five nucleotides long. GC clamps at the three-prime end are good, but long runs of G or C should be avoided to prevent non-specific binding. The statement that the melting temperatures must differ by at least ten degrees is incorrect; for efficient amplification, the forward and reverse primers should have melting temperatures that are as close as possible.

**246.** (C) Absolute quantification requires a serially diluted standard curve of known concentration.
Relative quantification in qPCR measures the change in expression of a gene compared to a control, usually by normalizing to a housekeeping gene. In contrast, absolute quantification is used when the exact number of copies must be known. This requires the creation of a standard curve using a template whose concentration has been precisely determined. The quantity in the unknown samples is then calculated by comparing their Ct values to this known standard.

**247.** (D) Short double-stranded oligonucleotides with restriction site.
Linkers are essential tools for cloning cDNA into vectors. Since cDNA synthesized in the lab is typically blunt-ended, it is difficult to ligate into vectors with high efficiency. Linkers are short, chemically synthesized, double-stranded DNA molecules that contain a specific recognition sequence for a restriction enzyme. Once these are ligated to the ends of the cDNA and then digested, they provide the cohesive sticky ends needed for successful cloning into a vector.

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signal to be produced, the antigen must possess at least two distinct epitopes. This "double-check" significantly reduces the chance of false positives and ensures that only the intended target is quantified.

**232.** (A) Recognized as foreign, triggering a HAMA response.
While mouse monoclonal antibodies are powerful tools in the laboratory, they have a major limitation for human therapy. Because they are mouse proteins, the human immune system recognizes them as foreign invaders. This triggers a "human anti-mouse antibody" response, where the patient's immune system attacks and neutralizes the therapeutic antibody. This can lead to serious allergic reactions and prevents the antibody from reaching its target and functioning effectively.

**233.** (B) Ensure penetration of membranes during hybridization.
For fluorescence in situ hybridization to work, the labeled DNA probe must be able to move into the nucleus and reach the target chromosomes. Intact chromosomes and nuclear membranes are complex structures that can act as a barrier to large molecules. By using probes that are fragmented into smaller pieces, typically between one hundred and five hundred base pairs, researchers ensure that the probe can easily penetrate these structures and hybridize efficiently.

**234.** (B) Enter the pores, traversing a longer path, and elute later.
Gel filtration chromatography separates molecules based on how they interact with the porous beads in the column. Large molecules are excluded from the beads and take the fastest route. Small molecules, however, are small enough to enter the pores. This means they must travel through the internal volume of all the beads as they move down the column. This longer, more circuitous path slows them down, causing them to elute later.

**235.** (A) Increases linearly with the percentage of G-C pairs
The melting temperature of DNA is the point at which the two strands are separated. Because each guanine-cytosine base pair is held together by three hydrogen bonds, while adenine-thymine pairs have only two, the G-C pairs are more stable. This structural difference means that as the percentage of G-C pairs in a DNA fragment increases, the total energy required to denature the molecule also increases, resulting in a linear rise in the melting temperature.

NODIA

**236.** (B) Causes severe chemical burns and acts as a potent toxin.
Phenol is one of the most hazardous chemicals used in the molecular biology laboratory. It is a powerful organic solvent that can rapidly penetrate the skin. Once in contact, it causes severe and deep chemical burns, often without immediate pain because it also has an anesthetic effect. Furthermore, phenol is a systemic toxin that can be absorbed into the bloodstream, potentially leading to damage to the liver, kidneys, and central nervous system.

**237.** (D) Cation bridges (utilizing sodium or guanidinium ions).
The purification of DNA on a silica column is based on the chemical interaction between the negatively charged silica surface and the negatively charged phosphate backbone of the DNA. Under normal conditions, these two would repel each other. However, in the presence of high concentrations of chaotropic salts, monovalent cations like sodium or guanidinium act as "bridges." They neutralize the charges and allow the DNA to bind tightly to the silica.

**238.** (A) P-4, Q-2, R-1, S-3
Illumina sequencing is based on imaging reversible fluorescent dye-terminator nucleotides as they are incorporated. Ion Torrent uses semiconductor sensors to detect pH changes caused by hydrogen ion release. PacBio SMRT sequencing utilizes zero-mode waveguides for the real-time detection of fluorescent pulses from single molecules. Finally, pyrosequencing identifies the DNA sequence by detecting the visible light produced through an enzymatic cascade initiated by the release of pyrophosphate.

**239.** (B) RNA is highly pure and largely free of protein.
When measuring the purity of an RNA sample using spectrophotometry, the $\frac{A_{260}}{A_{280}}$ ratio is the standard indicator. While pure DNA has a ratio of 1.8, pure RNA typically has a higher ratio of approximately 2.0. A measured value of 1.95 is very close to this ideal, indicating that the extraction was successful and the sample is

**223.** (B) Primers have partial complementarity, allowing hybridization.
A primer dimer is a common artifact in PCR that appears as a small, low molecular weight band. It occurs when the two primers in the reaction have sequences that are partially complementary to each other, particularly at their three-prime ends. This allows the primers to anneal to one another instead of the template DNA. The DNA polymerase then extends these annealed primers, creating a short, double-stranded product that is further amplified.

**224.** (A) II, III, IV and V
Successful genomic libraries require high-capacity vectors like BACs to manage large eukaryotic genomes, T4 DNA ligase for joining fragments, and alkaline phosphatase to prevent the vector from simply closing back on itself. These libraries represent the entire genome, including introns and exons. Statement I is incorrect because complete digestion is avoided; instead, partial digestion is used to create overlapping fragments and ensure a representative collection of genes.

**225.** (A) The types and relative abundances of the represented cloned sequences
Every cell in an organism contains the same genomic DNA, but different tissues express different sets of genes. Since a cDNA library is created from the messenger RNA present in a tissue, it serves as a snapshot of the active genes. A liver cDNA library will be rich in transcripts for metabolic enzymes, while a brain library will contain more transcripts related to neurotransmission, reflecting their different biological functions.

**226.** (C) It induces the expression of the cloned gene by de-repressing the lac promoter.
In immunological screening, the goal is to have the bacterial host produce the protein encoded by the cloned cDNA. Many expression vectors use the lac promoter to control this process. IPTG is a structural analog of lactose that is not metabolized by the bacteria. When added to the culture, it binds to the lac repressor, causing it to release the DNA and allowing the bacterium to transcribe the cloned gene.

**227.** (A) Multiple unique restriction sites located within a selectable marker gene
An ideal cloning vector must have several key features to be useful. One of the most important is a "multiple cloning site," which is a small region containing many unique restriction sites where foreign DNA can be inserted. This site is usually located within a selectable marker gene, such as lacZ'. This allows researchers to easily identify recombinant clones through methods like blue-white screening based on the disruption of the gene.

**228.** (D) Pyrosequencing
Pyrosequencing is a next-generation sequencing platform that monitors the incorporation of nucleotides in real-time. Each time a base is added, a molecule of pyrophosphate is released. This pyrophosphate is used in a multi-enzyme cascade that ultimately results in the conversion of luciferin to oxyluciferin. This reaction produces a flash of visible light, which is detected and used to determine the DNA sequence as each base is provided.

**229.** (A) To provide essential growth factors and support cell viability at low densities.
During the final stages of monoclonal antibody production, individual hybridoma cells are isolated by limiting dilution. Because these cells are often fragile and do not grow well when they are alone in a well, a "feeder layer" of other cells, such as peritoneal macrophages, is often added. These feeder cells do not divide themselves but secrete essential growth factors and cytokines that help the single hybridoma cell survive and multiply.

**230.** (B) III only
Analytical ultracentrifuges use real-time optics, and rate-zonal centrifugation uses density gradients. The Svedberg unit is indeed a measure of time, and CsCl is standard for DNA gradients. However, the statement that isopycnic centrifugation separates particles purely based on molecular weight is incorrect. Isopycnic centrifugation separates particles exclusively according to their buoyant density, regardless of their total mass or size, once they reach their equilibrium position in the gradient.

**231.** (D) Antigen must possess at least two distinct epitopes.
A sandwich ELISA is generally considered the most specific ELISA format. This is because it requires the target antigen to be recognized and bound by two different antibodies: the capture antibody and the detection antibody. For a

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**248.** (A) Both A and R are true and R is the correct explanation of A.
Many parts of the genome contain long stretches of repetitive DNA that are difficult to sequence using short-read technologies like Illumina because the reads cannot span the entire repeat. PacBio SMRT sequencing is uniquely capable of solving this problem. Because it generates extremely long individual reads, often exceeding ten or twenty kilobases, it can easily read through these repetitive regions, providing the necessary data for high-quality de novo genome assembly and structural analysis.

**249.** (B) Safer to handle, longer shelf life, allow multiplexing.
Traditional in situ hybridization used radioactive probes, which were hazardous, had a short shelf life, and required long exposure times. Fluorescent labels in FISH offer several major advantages. They are much safer to handle in the laboratory and are stable for long periods. Furthermore, because many different fluorescent colors are available, researchers can use multiple probes in the same sample to detect and distinguish several different target sequences at the same time.

NODIA

**250.** (A) I, III and IV
Analytical ultracentrifugation is a sophisticated technique that uses very small sample volumes and specialized rotors with optical windows to monitor the sedimentation process in real-time. Sedimentation velocity experiments are a primary application, used to measure how fast molecules move in response to gravity. Statement II is incorrect because it is actually a powerful tool for determining molecular weight. Statement V is also incorrect, as it is used for characterization, not large-scale cell purification.

**********

1. End-filling of sticky ends
2. Nick sealing using ATP
3. 5' end labeling
4. Prevention of self-ligation

(A) P-2, Q-4, R-3, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-4, Q-2, R-1, S-3
(D) P-1, Q-4, R-2, S-3

2012

**21.** Which enzyme is most appropriate for blunt-ending DNA with 3' overhangs?
(A) Taq polymerase
(B) T4 Polynucleotide Kinase
(C) T4 DNA polymerase
(D) DNA ligase

2004

**22.** In the exchange reaction catalyzed by T4 PNK, what occurs in the presence of excess ADP?
(A) Direct phosphorylation of available 3' termini
(B) Gamma-phosphate exchange at 5' ends
(C) Terminal dephosphorylation without replacement
(D) Non-specific processing as an exonuclease

2019

**23.** Plasmid pBR322 carries two distinct antibiotic resistance genes, which are:
(A) Ampicillin and Kanamycin
(B) Kanamycin and Chloramphenicol
(C) Tetracycline and Streptomycin
(D) Ampicillin and Tetracycline

2017

**24.** Which of the following is the correct statement regarding the pUC19 vector?
(A) MCS located within the lacZ' gene
(B) Inheritance of a very low copy number origin
(C) Dual resistance to ampicillin and tetracycline
(D) Total inability to support α-complementation

2007

**25.** The replication origin (ori) in plasmids like pBR322 is derived from which naturally occurring plasmid?
(A) Ti plasmid
(B) pSC101
(C) ColE1
(D) F plasmid

2012

**26.** Identify the INCORRECT statement regarding plasmid incompatibility.
(A) Plasmids with different replication origins are typically incompatible
(B) Plasmids from the same incompatibility group cannot stably co-exist in the same host cell
(C) Incompatibility is determined by the mechanism of replication regulation
(D) pBR322 and pUC19 share the same incompatibility group

2002

**27.** In the ColE1 replication system, which molecule acts as an antisense inhibitor to regulate copy number?
(A) RNA II
(B) Rop protein exclusively
(C) Rom transcript
(D) RNA I

2014

**28.** Which component is absent in the pUC19 vector, leading to its significantly higher copy number compared to pBR322?
(A) RNA I
(B) Rop gene
(C) RNA II
(D) Ampicillin resistance gene

2008

**29.** The pGEM-T vector system is specifically designed for cloning PCR products and relies on the presence of single:
(A) 5' G overhangs
(B) 3' C overhangs
(C) 3' T overhangs
(D) 5' T overhangs

2004

**30.** Consider the following statements regarding plasmid vectors:
I. pBR322 was artificially constructed from multiple natural plasmids.
II. In pBR322, cloning into the BamHI site disrupts ampicillin resistance.
III. pUC19 utilizes blue-white screening for recombinant selection.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I only

2014

**31.** If two plasmids share the same repressor control system for replication, they will exhibit:
(A) Incompatibility
(B) Symbiosis

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2014

**10.** Given below are two statements: one is labeled as Assertion (A) and the other is labeled as Reason (R).
Assertion (A): Type II restriction enzymes are the primary tools used in gene cloning.
Reason (R): They recognize specific palindromic sequences and cleave strictly within or very near those sites without requiring ATP.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2017

**11.** In the nomenclature of restriction enzyme EcoRI, the "R" represents the:
(A) Genus
(B) Species
(C) Order of discovery
(D) Strain

2006

**12.** T4 DNA ligase catalyzes the formation of phosphodiester bonds. Which cofactor is strictly required for this reaction?
(A) $NAD^+$
(B) ATP
(C) GTP
(D) FAD

2004

**13.** Unlike T4 DNA ligase, E. coli DNA ligase requires a different cofactor for sealing nicks in double-stranded DNA. What is it?
(A) ATP
(B) SAM
(C) $NAD^+$
(D) NADH

2021

**14.** What is the primary function of Calf Intestinal Phosphatase (CIP) in cloning procedures?
(A) Addition of phosphate groups to 5' termini
(B) Repair of mismatched or damaged nucleotides
(C) Cleavage of internal phosphodiester RNA bonds
(D) Removal of terminal 5' phosphate groups

2007

**15.** The Klenow fragment of E. coli DNA Polymerase I retains which of the following enzymatic activities?
(A) 5' to 3' polymerase and 3' to 5' exonuclease
(B) 5' to 3' exonuclease and 5' to 3' polymerase
(C) 5' to 3' exonuclease only
(D) 3' to 5' exonuclease only

2001

**16.** What is a practical advantage of using Shrimp Alkaline Phosphatase (SAP) over CIP?
(A) Total lack of requirement for metallic cofactors
(B) Simple and permanent heat-inactivation
(C) Significant levels of intrinsic DNA ligase activity
(D) High catalytic efficiency under acidic pH conditions

2019

**17.** T4 Polynucleotide Kinase (PNK) is primarily used in molecular cloning for:
(A) Enzymatic removal of terminal phosphate groups
(B) Phosphorylation of 5' hydroxyl termini
(C) De novo synthesis of short RNA primer sequences
(D) Targeted degradation of single-stranded DNA ends

NODIA

2010

**18.** Consider the following statements about the Klenow Fragment:
I. It is produced by proteolytic cleavage of DNA Polymerase I.
II. It is used to fill in 5' recessed termini.
III. It possesses a 5' to 3' exonuclease activity.
Which of the above statement(s) is/are correct?
(A) I and III
(B) II and III
(C) I and II
(D) I only

2024

**19.** During the reaction mechanism of DNA ligase, which transient intermediate is formed before the final phosphodiester bond closure?
(A) Enzyme-NADH complex
(B) Enzyme-GMP intermediate
(C) Enzyme-AMP intermediate
(D) DNA-AMP intermediate

2003

**20.** Consider the following matching pairs:
List I: (Enzyme)
P. T4 DNA Ligase
Q. Alkaline Phosphatase
R. Polynucleotide Kinase
S. Klenow Fragment
List II: (Function)

**206.** (C) Skewed towards dNTPs, to allow extension before termination.
In a Sanger sequencing reaction, there must be a careful balance between the regular deoxynucleotides (dNTPs) and the chain-terminating dideoxynucleotides (ddNTPs). If the concentration of ddNTPs is too high, every strand will terminate immediately. The ratio is heavily skewed toward the regular dNTPs, typically around 100:1. This ensures that the polymerase can extend the chain for hundreds of bases before randomly incorporating a ddNTP and terminating the fragment.

**207.** (B) The release of a hydrogen ion ($H^+$) resulting in a pH change
Most next-generation sequencing technologies, like Illumina, rely on the detection of light or fluorescent signals. Ion Torrent is unique because it uses a semiconductor-based approach to measure chemical changes. Each time a DNA polymerase adds a nucleotide to a growing strand, a hydrogen ion is released. This ion causes a tiny change in the pH of the surrounding solution, which is detected and recorded as an electronic signal.

**208.** (D) The Fc region of the primary antibody
The success of an indirect ELISA depends on the ability of the secondary antibody to find and bind to the primary antibody. Secondary antibodies are produced to recognize the constant, or Fc, region of antibodies from a different species. For example, if the primary antibody was made in a mouse, a "goat anti-mouse" secondary antibody is used. This secondary antibody is conjugated to an enzyme to provide the detection signal.

**209.** (A) Highly susceptible to cross-reactivity and variation.
Polyclonal antibodies are a mixture of antibodies produced by different B-cell lineages, meaning they recognize many different parts of an antigen. While this can increase sensitivity, it also increases the risk that some antibodies in the mix will bind to similar proteins, causing cross-reactivity. Furthermore, because they are harvested from animal serum, there is significant variation between different animals and different batches, making them less consistent than monoclonal antibodies.

**210.** (A) Both A and R are true and R is the correct explanation of A.
A sandwich ELISA requires two different antibodies to bind to the same antigen simultaneously. This is often impossible for small molecules or haptens because they do not have enough surface area for two large antibody molecules to attach. Competitive ELISA is the ideal solution for these small targets. In this format, the small antigen from the sample only needs to compete for a single antibody binding site, making it highly effective.

**211.** (A) Less radiolabeled antigen binding to the antibody
Radioimmunoassay is a competitive assay where a fixed amount of radiolabeled antigen and a sample of unlabeled antigen compete for a limited number of antibody binding sites. When the amount of unlabeled antigen in the sample is increased, it occupies a larger share of the available antibodies. As a result, the amount of the radiolabeled tracer that can bind to the antibodies decreases, which is how the sample concentration is measured.

**212.** (D) Spectral Karyotyping or Multiplex FISH.
Standard FISH usually identifies only one or two specific sequences. Spectral Karyotyping, or Multiplex FISH, is an advanced variation that uses a combination of five or more different fluorophores. By labeling each chromosome with a unique blend of these colors, researchers can "paint" every chromosome in the cell a different color. This allows for the easy identification of complex chromosomal rearrangements and translocations across the entire genome.

**213.** (B) Whole cells and nuclei
Differential centrifugation separates cellular components based on how fast they move in a centrifugal field, which is determined by their size and density. Whole cells and nuclei are the largest and densest organelles in a typical eukaryotic cell. Therefore, they have the highest sedimentation rate and will be the first components to settle and form a pellet at the lowest speeds, such as six hundred times gravity.

**214.** (C) Biological recognition (e.g., enzyme-substrate).
Affinity chromatography is the most selective form of chromatography, as it exploits the natural, highly specific interactions found in

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(C) Conjugation
(D) Recombination

2025

**32.** In pUC19, where is the origin of replication (ori) located relative to the antibiotic resistance gene?
(A) Physical overlap with the ampicillin gene
(B) They exist as separate structural units
(C) Location of ori within the lacZ' sequence
(D) Function as a promoter for antibiotic genes

2023

**33.** Which of the following is NOT a functional feature of the pBR322 vector?
(A) Origin of replication
(B) A-complementation region
(C) Selectable markers
(D) Multiple unique restriction sites

2013

**34.** The theoretical maximum size limit for foreign DNA packaging into a typical wild-type Lambda phage head is approximately:
(A) 52 kb
(B) 25 kb
(C) 10 kb
(D) 85 kb

2021

**35.** Identify the correct distinction between Lambda insertion and replacement vectors.
(A) Replacement vectors are typically smaller than insertion vectors
(B) Insertion vectors can accommodate much larger DNA fragments than replacement vectors
(C) Replacement vectors contain a stuffer fragment that is discarded during cloning
(D) λgt10 is an example of a replacement vector

2012

**36.** In the λgt10 insertion vector, the unique restriction site used for cloning is located within which gene, enabling selection?
(A) lacZ
(B) ampR
(C) tetR
(D) cI (repressor)

2003

**37.** λEMBL4 is widely used for constructing genomic libraries. What is its approximate cloning capacity?
(A) 1-5 kb
(B) 5-10 kb
(C) 9-23 kb
(D) 35-45 kb

2015

**38.** M13 phagemid systems are unique because the viral DNA isolated from intact viral particles is:
(A) Double-stranded linear
(B) Single-stranded circular
(C) Double-stranded circular
(D) Single-stranded linear

2025

**39.** When utilizing a phagemid vector like pBluescript, what is strictly required to package the single-stranded DNA into viral particles?
(A) Saturation with high calcium ions
(B) Specialized heat shock treatment
(C) Use of in vitro packaging extracts
(D) Infection with a helper phage

2009

**40.** Consider the following statements concerning phagemids:
I. They carry both a plasmid and a phage origin of replication.
II. They encode all structural proteins needed for phage assembly.
III. They can be propagated as double-stranded plasmids in E. coli.
IV. pBluescript is a common example.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II and III
(D) I, II and III

2011

**41.** Identify the INCORRECT statement regarding M13 bacteriophage biology.
(A) Production of filamentous M13 virions
(B) Double-stranded replicative form (RF)
(C) Host cell lysis and subsequent death
(D) Viral extrusion through the cell envelope

2021

**42.** The pBluescript phagemid contains promoters flanking the multiple cloning site to allow in vitro transcription. These are typically:
(A) T3 and T7 promoters
(B) SP6 and CMV promoters
(C) CaMV 35S promoters
(D) lac and trc promoters

2009

**43.** Which of the following is ABSENT in commercial bacteriophage in vitro packaging extracts?
(A) Assembled phage head precursors

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(B) Two identical 25 bp inverted repeats
(C) Two distinct 100 bp palindromes
(D) 25 bp imperfect direct repeats (Right and Left borders)

2018

**68.** The primary distinction between binary and co-integrate vector systems for Agrobacterium is that in binary systems:
(A) The T-DNA lacks border sequences
(B) The vir genes and T-DNA are located on separate plasmids
(C) Recombination between the helper and T-DNA plasmids must occur
(D) Two distinct bacterial strains are required

2003

**69.** pBIN19 is a classic example of a:
(A) Co-integrate vector
(B) Helper plasmid
(C) Binary vector
(D) High-capacity eukaryotic vector

2006

**70.** Consider the following matching pairs:
List I: (Protein)
P. VirA
Q. VirD2
R. VirE2
S. VirB
List II: (Function)
1. Single-stranded DNA binding protein
2. Endonuclease that cuts T-DNA borders
3. Sensor kinase for plant phenolics
4. Type IV secretion system component
(A) P-3, Q-2, R-1, S-4
(B) P-2, Q-1, R-4, S-3
(C) P-3, Q-1, R-2, S-4
(D) P-1, Q-3, R-4, S-2

2022

**71.** Identify the correct statement regarding T-DNA processing in Agrobacterium.
(A) Absolute requirement of the left border
(B) Right border cleavage by VirE2 protein
(C) Double-stranded transfer of the T-DNA
(D) Covalent 5' attachment of VirD2

2011

**72.** The pGreen series of binary vectors differs from pBIN19 because it relies on a mutant RepA protein provided by:
(A) A helper plasmid named pSoup
(B) The endogenous host plant genome
(C) Native E. coli DNA polymerase
(D) Agrobacterium chromosomal genes

2005

**73.** Which of the following is fundamentally EXCLUDED from the transferred T-DNA sequence in engineered binary vectors?
(A) Selectable marker gene
(B) Promoter sequences
(C) Gene of interest
(D) vir gene operon

2019

**74.** Which natural phenolic compound released by wounded plant tissues acts as a potent inducer of the Agrobacterium vir operon?
(A) Acetosyringone
(B) Anthocyanin
(C) Salicylic acid
(D) Jasmonic acid

2022

**75.** In a binary vector system, the helper Ti plasmid possesses which of the following?
(A) The gene of interest only
(B) Both T-DNA borders and vir genes
(C) vir genes but no T-DNA borders
(D) T-DNA borders but no vir genes

2016

**76.** Identify the INCORRECT statement regarding a "disarmed" Ti plasmid.
(A) It does not cause crown gall disease
(B) It lacks the essential vir genes for transfer
(C) It lacks the oncogenes responsible for auxin and cytokinin synthesis
(D) It maintains the ability to facilitate gene transfer

2021

**77.** The "overdrive" sequence found adjacent to the right border in some Ti plasmids functions to:
(A) Suppress vir gene expression
(B) Replicate the T-DNA in the plant nucleus
(C) Trigger apoptosis in the plant cell
(D) Enhance the efficiency of T-DNA transfer

NODIA

2025

**78.** When preparing chemically competent E. coli cells using $CaCl_2$, the exact mechanism of competence induction primarily involves:
(A) Shielding of electrostatic repulsion
(B) Direct enzymatic digestion of walls
(C) Complete removal of membrane lipids
(D) Covalent bonding of calcium to DNA

2004

**79.** During the chemical transformation protocol, the brief heat shock step (e.g., $42^{\circ}C$ for 45s) serves to:

2015

**56.** The tac promoter used in E. coli expression vectors is a strong hybrid promoter derived from:
(A) T7 and T3 promoters
(B) trp and lac promoters
(C) tet and lac promoters
(D) trp and ara promoters

2003

**57.** High-level expression driven by the T7 promoter requires the host E. coli to express which specialized enzyme?
(A) T7 DNA polymerase
(B) T7 RNA polymerase
(C) Taq polymerase
(D) T4 RNA ligase

2016

**58.** Identify the correct distinction between Shine-Dalgarno (SD) and Kozak sequences.
(A) SD for prokaryotes; Kozak for eukaryotes
(B) SD in eukaryotes; Kozak in prokaryotes
(C) Shared roles as transcriptional promoters
(D) Binding sites for DNA and RNA polymerases

2016

**59.** The Glutathione S-transferase (GST) tag is a popular translational fusion tag because it allows single-step purification using:
(A) Nickel-NTA agarose
(B) Amylose resin
(C) Glutathione-Sepharose beads
(D) Glutathione-coated chitin beads

2022

**60.** Given below are two statements: one is labeled as Assertion (A) and the other is labeled as Reason (R).
Assertion (A): Hexahistidine (His-tag) fusions enable efficient protein purification through Immobilized Metal Affinity Chromatography (IMAC).
Reason (R): The consecutive histidine residues exhibit strong affinity for transition metal ions like $Ni^{2+}$ or $CO_2^+$ immobilized on a matrix.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2013

**61.** Identify the INCORRECT statement concerning the Maltose Binding Protein (MBP) fusion tag.
(A) It is often used to enhance the solubility of recombinant proteins
(B) It requires nickel affinity chromatography for purification
(C) It can be cleaved off using specific proteases
(D) It binds to amylose resin for purification

2018

**62.** The CaMV 35S promoter is heavily utilized in transgenic research. It is categorized as a:
(A) Constitutive plant promoter
(B) Tissue-specific plant promoter
(C) Inducible prokaryotic promoter
(D) Repressible fungal promoter

2022

**63.** Which of the following is generally LEAST relevant for building a eukaryotic expression cassette?
(A) Enhancer elements
(B) Polyadenylation signal
(C) Shine-Dalgarno sequence
(D) Kozak sequence

2025

**64.** The Shine-Dalgarno sequence typically relies on base-pairing with which ribosomal component during translation initiation?
(A) 23S rRNA of the 50S subunit
(B) 18S rRNA of the 40S subunit
(C) 5S rRNA of the 50S subunit
(D) 16S rRNA of the 30S subunit

2024

**65.** The lac promoter is a classical inducible promoter. Which molecule naturally binds the lac repressor to induce expression?
(A) Glucose
(B) Allolactose
(C) cAMP
(D) ATP

2025

**66.** To separate a fusion tag from the target recombinant protein, an expression vector must include a specific sequence between the tag and the gene. This sequence encodes:
(A) A stop codon
(B) A protease cleavage site
(C) An internal ribosome entry site (IRES)
(D) A poly-A tail

2001

**67.** The T-DNA region of the wild-type Agrobacterium tumefaciens Ti plasmid is defined by:
(A) Two identical 25 bp direct repeats

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(D) Deoxythymidine (T)

2018

**114.** Identify the correct statement regarding blunt-end ligation.
(A) It requires significantly lower concentrations of DNA ligase compared to sticky-end ligation
(B) It has a higher efficiency than cohesive-end ligation
(C) It inherently guarantees directional cloning
(D) Transient base-pairing does not occur to stabilize the ends before ligation

2021

**115.** What distinguishes a DNA linker from an adapter in molecular cloning?
(A) Adapters have pre-formed sticky ends
(B) Use of single versus double strands
(C) Differential requirement for ligation
(D) Composition consisting of RNA molecules

2001

**116.** Which of the following is fundamentally bypassed when utilizing Gateway recombinational cloning technology?
(A) Requirement for host selectable markers
(B) Restriction and ligation steps
(C) Propagation within E. coli host cells
(D) Initial amplification of genes via PCR

2001

**117.** In the Gateway cloning system, the BP clonase enzyme mix catalyzes recombination between which two specific attachment (att) sites?
(A) attL and attR
(B) attB and attL
(C) attP and attR
(D) attB and attP

2009

**118.** The creation of an Expression Clone from an Entry Clone in Gateway technology requires the LR clonase enzyme mix, which acts on:
(A) attB and attP sites
(B) loxP and FRT sites
(C) cos and loxP sites
(D) attL and attR sites

2016

**119.** To eliminate un-recombined Destination vectors in Gateway LR reactions, the Destination vector uniquely contains which lethal gene between the attR sites?
(A) nptII
(B) sacB
(C) ccdB
(D) ccdA

2018

**120.** Consider the following matching pairs:
List I: (Cloning Technique)
P. TA Cloning
Q. Directional Cloning
R. Blunt-end Ligation
S. Gateway Cloning
List II: (Key Feature)
1. Uses site-specific recombination
2. Relies on single 3'-A overhangs
3. Uses two distinct restriction enzymes
4. Lower efficiency, requires high T4 ligase
(A) P-2, Q-3, R-4, S-1
(B) P-3, Q-2, R-1, S-4
(C) P-4, Q-1, R-3, S-2
(D) P-1, Q-4, R-2, S-3

2015

**121.** To prevent vector self-ligation during non-directional sticky-end cloning, the linearized vector is typically treated with:
(A) Exonuclease III
(B) T4 Polynucleotide kinase
(C) Alkaline phosphatase
(D) DNA polymerase

2013

**122.** During the construction of a cDNA library, eukaryotic mRNA is selectively isolated from total cellular RNA utilizing an affinity column containing:
(A) Oligo-dT cellulose
(B) Oligo-dG cellulose
(C) Poly-A binding protein
(D) Silica membrane

2002

**123.** The first-strand cDNA synthesis relies on reverse transcriptase. Which primer is most universally used to copy all mRNAs in a sample?
(A) Random hexamers
(B) Specific gene primers
(C) Oligo-dT
(D) Oligo-dC

2018

**124.** In the classical Gubler-Hoffman method for second-strand cDNA synthesis, what is the role of RNase H?
(A) Total degradation of mRNA templates
(B) Synthesis of the second DNA strand
(C) Ligation of newly synthesized DNA
(D) Nicking of the mRNA template

NODIA

(A) Denaturation of plasmids into strands
(B) Activation of chromosomal polymerases
(C) Thermal gradient-driven DNA uptake
(D) Inactivation of host restriction units

2005

**80.** Consider the following statements regarding electroporation of E. coli:
I. It relies on short, high-voltage electrical pulses.
II. High salt concentrations in the buffer improve transformation efficiency.
III. It creates transient pores in the bacterial cell membrane.
Which of the above statement(s) is/are correct?
(A) I and III
(B) II and III
(C) I and II
(D) I only

2006

**81.** The use of Rubidium Chloride (RbCl) in chemical competence protocols provides what specific advantage over standard $CaCl_2$?
(A) Higher transformation efficiency
(B) Elimination of heat shock steps
(C) Uptake of linear genomic DNA
(D) Targeting gram-positive species

2010

**82.** Identify the correct statement regarding the buffer requirements for electroporation.
(A) Requirement for high sample conductivity
(B) Use of high ionic strength buffer systems
(C) Use of low-salt or glycerol washes
(D) Presence of high EDTA concentrations

2025

**83.** The "time constant" in an electroporation experiment is defined by the circuit's:
(A) Voltage and current
(B) Capacitance and inductance
(C) Voltage and resistance
(D) Resistance and capacitance

2021

**84.** Which phenomenon must be strictly avoided during electroporation because it kills cells and destroys the sample?
(A) Arcing
(B) Osmotic shock
(C) Competence induction
(D) Depolarization

2003

**85.** Typical field strengths applied during E. coli electroporation using standard 1-mm or 2-mm cuvettes range around:
(A) 1-2 kV/cm
(B) 12-18 kV/cm
(C) 50-60 kV/cm
(D) 100-200 V/cm

2022

**86.** Identify the INCORRECT requirement for standard chemical preparation of competent E. coli.
(A) Harvesting cells during the mid-log phase
(B) Maintaining the cells at strictly $0^{\circ}C$ (on ice) during salt washing
(C) Rapidly warming the cells to $37^{\circ}C$ before adding DNA
(D) Using low-temperature centrifugation

2024

**87.** Why are bacteria harvested specifically in the logarithmic (log) phase for competent cell preparation?
(A) Complete absence of bacterial cell walls
(B) Secretion of protective polysaccharides
(C) Highly permeable growing membranes
(D) Autonomous replication of the plasmids

2008

**88.** Arcing during electroporation is most frequently caused by:
(A) Elevated sample salt concentration
(B) Application of insufficient voltage
(C) Excessive cell volume in the cuvettes
(D) Use of purified salt-free DNA samples

2014

**89.** In pBR322, cloning a fragment into the SalI restriction site abolishes the cell's resistance to tetracycline. This process is called:
(A) A-complementation
(B) Insertional inactivation
(C) Blue-white screening
(D) Positive selection

2024

**90.** Consider the following statements about blue-white screening:
I. It requires a host strain with a deleted lacZ $\alpha$ peptide sequence.
II. Recombinant colonies appear blue because $\beta$-galactosidase is active.
III. X-gal is the chromogenic substrate utilized.
IV. The multiple cloning site is located within the lacZ' sequence.

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2018

**136.** Identify the correct initial step uniquely required for chromosome jumping library construction but not for walking.
(A) DNA digestion into 1 kb fragments
(B) Transcription of total cellular RNA
(C) Random-primer PCR amplification
(D) Circularization of large fragments

2004

**137.** In chromosome jumping, after the large genomic DNA fragments are generated, they are circularized in the presence of:
(A) Use of selectable antibiotic markers
(B) Presence of reverse transcriptase
(C) A suppressor tRNA marker gene
(D) Conjugation with fluorescent tags

2025

**138.** Which of the following is NOT a characteristic of chromosome walking?
(A) It proceeds by using the end of one clone to probe a library for the next overlapping clone
(B) It requires the construction of restriction maps for each isolated clone
(C) It isolates only the extreme endpoints of a large region, discarding the middle
(D) It relies on sub-cloning fragment ends

2010

**139.** To generate a probe for the next step in a chromosome walk, one must precisely isolate a DNA sequence from:
(A) The center of the current clone
(B) The extreme distal end of the current clone
(C) The vector backbone
(D) The promoter region

2004

**140.** Consider the following steps in chromosome jumping:
I. Partial digestion to yield large fragments
II. Ligation of ends with a supF marker
III. Circularization of fragments under dilute conditions
IV. Cleavage with another restriction enzyme to isolate the marker-junction
Which of the above statement(s) represents essential steps in the process?
(A) I, II and IV
(B) II, III and IV
(C) I, II, III and IV
(D) I, III and IV

2024

**141.** Because chromosome walking in classical plasmid or phage libraries is tedious over long distances, modern walks predominantly utilize libraries built in:
(A) pUC19
(B) BACs or YACs
(C) M13 phagemids
(D) Expression vectors

2007

**142.** Identify the INCORRECT statement regarding library screening in chromosome walking.
(A) The probe must be completely free of repetitive sequences to avoid false positives
(B) It requires only one round of library screening to walk 1000 kb in a cosmid library
(C) Plaque hybridization or colony hybridization techniques are employed
(D) Overlapping positive clones must be restriction-mapped to establish contiguous alignment

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2007

**143.** The presence of the supF gene in a chromosome jumping vector allows for selection in specific E. coli hosts by:
(A) Provision of antibiotic resistance
(B) Fluorescence under ultraviolet light
(C) Amber nonsense mutation suppression
(D) Induction of lethal host cell lysis

2015

**144.** The initial step of Agrobacterium-mediated plant transformation involves bacterial chemotaxis toward wounded plant cells. This is mediated by plant-derived:
(A) Sugars and phenolic compounds
(B) Amino acids and lipids
(C) Alkaloids and terpenes
(D) Ethylene and abscisic acid

2008

**145.** In the Agrobacterium Virulence system, which two proteins function as a two-component regulatory system to sense phenolics and activate vir gene expression?
(A) VirD1 and VirD2
(B) VirB and VirD4
(C) VirE1 and VirE2
(D) VirA and VirG

2016

**146.** The transfer of the T-DNA complex from the bacterium into the plant cell cytoplasm occurs

2004

**102.** The hpt gene confers resistance to hygromycin B. What is the biochemical mechanism of the HPT enzyme?
(A) Aminoglycoside adenylation
(B) Aminoglycoside phosphorylation
(C) Aminoglycoside acetylation
(D) Ribosomal modification

2024

**103.** The bar gene, originally isolated from Streptomyces hygroscopicus, confers resistance to herbicides containing:
(A) Glyphosate
(B) 2,4-D
(C) Paraquat
(D) Phosphinothricin (glufosinate)

2017

**104.** Identify the correct action of phosphinothricin (the target of the bar gene) in non-transgenic plants.
(A) Inhibition of glutamine synthetase
(B) Competitive inhibition of EPSPS
(C) Blockage of PSII electron transport
(D) Inhibition of acetolactate synthase

2009

**105.** Phosphomannose isomerase (PMI) is used as a selectable marker in plants. Its mechanism of selection is fundamentally different because:
(A) Detoxification of lethal antibiotics
(B) Metabolic utilization of mannose
(C) Resistance to heavy metal toxicity
(D) Active export of cellular toxins

2004

**106.** Which of the following is NOT an example of a negative selectable marker (one that kills the organism expressing it)?
(A) Cytosine deaminase (codA)
(B) Diphtheria toxin A (DT-A)
(C) sacB
(D) Neomycin phosphotransferase (nptII)

2002

**107.** Hygromycin B toxicity in sensitive plant cells is caused by its interference with:
(A) DNA replication
(B) Photosynthesis
(C) Cell wall synthesis
(D) Protein translation

2012

**108.** The use of the pmi gene is considered "positive selection" (metabolic selection) rather than traditional selection because:
(A) Stimulation without toxic agents
(B) Reliance on antibiotic-based lethality
(C) Use of visual fluorescent markers
(D) Direct repair of damaged DNA strands

2016

**109.** Kanamycin inhibits bacterial and plant organelle function by specifically binding to:
(A) The 50S ribosomal subunit
(B) DNA gyrase
(C) The 30S ribosomal subunit
(D) RNA polymerase

2007

**110.** Given below are two statements: one is labeled as Assertion (A) and the other is labeled as Reason (R).
Assertion (A): PMI-based selection is often favored over antibiotic-based selection in modern crop biotechnology.
Reason (R): It avoids the bio-safety concerns related to the environmental release of antibiotic resistance genes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**111.** What is the primary advantage of directional cloning over non-directional cloning?
(A) It relies entirely on blunt-end ligation
(B) It avoids the need for DNA ligase
(C) It allows cloning without restriction enzymes
(D) It ensures the insert is integrated in the correct orientation

2017

**112.** TA cloning is a highly efficient method for cloning PCR products. It exploits the inherent terminal transferase activity of:
(A) Pfu DNA polymerase
(B) Taq DNA polymerase
(C) T4 DNA polymerase
(D) Klenow fragment

2017

**113.** In the TA cloning strategy, Taq polymerase preferentially adds a single non-templated nucleotide to the 3' ends of the PCR product. Which nucleotide is added?
(A) Deoxycytidine (C)
(B) Deoxyguanosine (G)
(C) Deoxyadenosine (A)

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(B) Essential viral tail proteins
(C) Catalytic DNA packaging enzymes
(D) Functional replication origins

2004

**44.** The specific sequence at the ends of the linear Lambda phage genome that allows it to circularize inside the host cell is known as:
(A) oriC
(B) cos site
(C) loxP site
(D) attB site

2019

**45.** Cosmid vectors combine features of plasmids and lambda phages. What is their typical cloning capacity?
(A) 1-5 kb
(B) 10-20 kb
(C) 35-45 kb
(D) 100-150 kb

2004

**46.** Yeast Artificial Chromosomes (YACs) require specific structural components to function. These include:
(A) Dedicated origins for replication
(B) Standard centromeres and sites
(C) Specific viral packaging signals
(D) CEN, TEL, and ARS components

2007

**47.** A major advantage of Fosmids over standard cosmids in library construction is:
(A) Stringent copy number regulation
(B) Absence of antibiotic resistance markers
(C) Capacity for fragments exceeding 150 kb
(D) Exclusion of in vitro packaging protocols

2017

**48.** Bacterial Artificial Chromosomes (BACs) are maintained stably in E. coli at a single copy per cell. They are primarily derived from:
(A) ColE1 plasmids
(B) F-factor plasmids
(C) Lambda phage
(D) M13 phage

2013

**49.** P1-derived Artificial Chromosomes (PACs) utilize the replication system of which bacteriophage?
(A) Bacteriophage Lambda
(B) Bacteriophage P1
(C) Bacteriophage M13
(D) Bacteriophage T4

2008

**50.** Consider the following vectors:
I. Plasmid pUC19
II. Cosmid
III. BAC
IV. YAC
Which of the following subsets is arranged in strict increasing order of cloning capacity?
(A) II, III, IV, I
(B) I, II, III, IV
(C) IV, III, II, I
(D) I, III, II, IV

2001

**51.** Identify the INCORRECT statement regarding YACs.
(A) Replication within eukaryotic host cells
(B) Dual markers for both yeast and bacteria
(C) Stability without chimeric artifacts
(D) Capacity for inserts larger than 500 kb

2004

**52.** How is recombinant BAC DNA most commonly introduced into E. coli host cells?
(A) Electroporation
(B) Calcium chloride heat shock
(C) In vitro packaging
(D) Agrobacterium infection

2008

**53.** If a researcher wishes to clone a contiguous 120 kb human genomic sequence stably in E. coli, which vector is most appropriate?
(A) Cosmid
(B) Plasmid pBR322
(C) BAC
(D) Lambda λgt10

2007

**54.** Which of the following is NOT a structural component typically found in a YAC cloning vector?
(A) URA3 marker
(B) TEL sequence
(C) CEN4 sequence
(D) cos site

NODIA

2018

**55.** The defining feature that allows a cosmid to be packaged into a phage head, unlike a standard plasmid, is the presence of:
(A) An F-factor origin
(B) Ampicillin resistance gene
(C) A cos site
(D) A centromere

2010

**125.** Following RNase H activity in the Gubler-Hoffman procedure, which enzyme is responsible for synthesizing the second DNA strand by nick translation?
(A) T4 DNA Polymerase
(B) E. coli DNA Polymerase I
(C) Reverse Transcriptase
(D) Taq DNA Polymerase

2021

**126.** Identify the correct distinction between a genomic library and a cDNA library from the same eukaryotic organism.
(A) Genomic libraries represent the entire genome, while cDNA libraries represent the transcriptome
(B) Inclusion of promoters and enhancers only occurs in cDNA libraries
(C) Representation of transcribed genes occurs exclusively in genomic libraries
(D) Significant reduction in library size is only seen in genomic libraries

2023

**127.** Which structural element of a gene would you NOT expect to find in a standard cDNA clone?
(A) 5' UTR (Untranslated Region)
(B) Coding Sequence (CDS)
(C) Poly-A tail
(D) Introns

2016

**128.** In vitro packaging is an essential step for constructing large genomic libraries using bacteriophage vectors. The packaging extracts provide:
(A) Empty phage heads and tail assemblies
(B) T4 DNA ligase
(C) Host E. coli cells
(D) Restriction enzymes

2021

**129.** To construct a representative genomic library, chromosomal DNA must be fragmented randomly into appropriately sized pieces. This is most commonly achieved by:
(A) Exhaustive frequent-cutter digestion
(B) Infrequent-cutter NotI digestion
(C) Partial Sau3AI digestion
(D) Heat-mediated boiling of DNA

2005

**130.** Consider the following steps involved in cDNA library construction:
I. mRNA isolation
II. RNase H and DNA Pol I treatment
III. Reverse transcription
Which of the following represents the correct chronological order of these steps?
(A) I, II and III
(B) II, I and III
(C) I, III and II
(D) III, I and II

2001

**131.** When cloning cDNA using EcoRI linkers, what vital step must be performed before adding the linkers to protect internal restriction sites?
(A) Treatment with EcoRI methylase
(B) Treatment with S1 nuclease
(C) Treatment with T4 DNA ligase
(D) Treatment with alkaline phosphatase

2022

**132.** The Clarke-Carbon equation, $N = \frac{\ln(1-P)}{\ln(1-f)}$, is utilized in library construction to determine the:
(A) Amount of vector DNA required for ligation
(B) Number of clones required for complete genome coverage
(C) Rate of specific recombination events
(D) Copy numbers of plasmids within hosts

2005

**133.** Chromosome walking is a molecular technique utilized primarily for:
(A) Identification of exact point mutations
(B) Copy number determination of target genes
(C) Mapping of transcription factor sites
(D) Spanning of uncharted DNA regions

2016

**134.** A severe limitation of standard chromosome walking is encountered when traversing:
(A) Arrays of repetitive sequences
(B) Proximity to highly transcribed genes
(C) Traversing of intron-rich regions
(D) Navigation of telomeric regions solely

2002

**135.** Chromosome jumping was developed specifically to overcome the limitations of chromosome walking by:
(A) Removal of genomic library requirements
(B) Bypassing repetitive DNA regions
(C) Utilization of cDNA instead of genomic DNA
(D) Simultaneous sequencing of whole genomes

NODIA

Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I and II

2012

**91.** The ccdB gene is used in positive selection vectors. How does it function?
(A) Resistance to chloramphenicol antibiotics
(B) Action as a fluorescent reporter system
(C) Poisoning of host DNA gyrase
(D) Production of a blue pigment when active

2016

**92.** Identify the correct principle of α-complementation.
(A) Dual provision of intact enzymes
(B) Vector-encoded complete lacZ genes
(C) Peptide complementation of lacZ
(D) Host-encoded α-peptide segments

2005

**93.** What is the role of X-gal in the blue-white screening process?
(A) Direct induction of the lac promoter
(B) Provision of antibiotic resistance markers
(C) Chromogenic lactose analog
(D) Inhibition of host cell RNA polymerase

2002

**94.** Which of the following is NOT typically utilized as a reporter gene in recombinant DNA technology?
(A) uidA (GUS)
(B) gfp
(C) luc (Luciferase)
(D) EcoRI

2009

**95.** The GUS reporter system, commonly used in plant transgenics, detects expression through the cleavage of which substrate to produce a blue precipitate?
(A) X-gal
(B) ONPG
(C) X-Gluc
(D) MUG

2005

**96.** The Green Fluorescent Protein (GFP), a popular in vivo reporter, was originally isolated from:
(A) E. coli
(B) Aequorea victoria
(C) Agrobacterium tumefaciens
(D) Thermus aquaticus

2009

**97.** Luciferase is often employed as a reporter for highly sensitive quantitative assays. Which of the following is required for its light-emitting reaction?
(A) X-Gluc and $Mg^{2+}$
(B) Luciferin, ATP, and $O_2$
(C) Calcium and calreticulin
(D) Hydrogen peroxide and luminol

2021

**98.** Identify the INCORRECT statement regarding positive selection systems.
(A) Only cells taking up a recombinant plasmid can survive
(B) They eliminate the need for secondary screening like blue-white selection
(C) Disruption of a lethal gene (like sacB or ccdB) permits host survival
(D) The lethal gene is activated specifically by the insertion of foreign DNA

2007

**99.** In blue-white screening, what is the specific role of IPTG?
(A) Use as a chromogenic dye substrate
(B) Inhibition of bacterial growth rates
(C) Induction of the lac promoter
(D) Use as a metabolic carbon source

2018

**100.** Consider the following selectable marker genes and their corresponding resistance profiles:
I. nptII (Kanamycin)
II. hpt (Hygromycin)
III. bar (Phosphinothricin)
IV. pat (Bialaphos)
V. cat (Chloramphenicol)
Which of the above paired combinations represents established resistance functions?
(A) I, II, III and V
(B) I, II, III and IV
(C) II, III, IV and V
(D) I, II, III, IV, and V

2018

**101.** The nptII gene is a ubiquitous selectable marker in plant transformation. It confers resistance by:
(A) Cleavage of the β-lactam rings
(B) Direct acetylation of the drug
(C) Phosphorylation of aminoglycosides
(D) Active export of antibiotic molecules

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via a Type IV secretion system primarily composed of:
(A) VirA proteins
(B) VirB operon proteins and VirD4
(C) VirC proteins
(D) VirG proteins

2016

**147.** Identify the correct function of the VirE2 protein during Agrobacterium infection.
(A) Protection of the single T-strand
(B) Covalent attachment to 5' termini
(C) Cleavage of the right T-DNA border
(D) Synthesis of complementary DNA

2022

**148.** Which host plant protein interacts with VirE2 to facilitate the nuclear import of the T-complex?
(A) Actin
(B) Rubisco
(C) Histone H3
(D) VIP1

2019

**149.** Which sequence is absolutely required in cis for the mobilization of T-DNA, yet is NOT transferred to the plant genome itself?
(A) Left border
(B) Right border
(C) vir operon
(D) Overdrive sequence

2015

**150.** Consider these steps of Agrobacterium-mediated transformation:
I. Sensing of acetosyringone by VirA
II. Phosphorylation of VirG
III. T-DNA processing by VirD1/VirD2
IV. Export via VirB/D4 pore
V. Nuclear import mediated by NLS signals
Which of the following lists these events in the correct chronological order?
(A) I, III, II and IV
(B) II, I, IV and V
(C) I, II, III, IV and V
(D) III, IV, I, II and V

2019

**151.** The integration of the T-DNA into the plant host genome relies primarily on which host cellular DNA repair mechanism?
(A) Homologous recombination (HR)
(B) Mismatch repair (MMR)
(C) Non-homologous end joining (NHEJ)
(D) Base excision repair (BER)

2004

**152.** Supervirulent Agrobacterium strains (e.g., EHA105) often harbor an extra plasmid carrying additional copies of which vir genes to boost transformation efficiency?
(A) virA/virG or virG/virE
(B) virB/virD4
(C) virC/virD1
(D) virD2/virE2

2001

**153.** What is the role of wound-response compounds like sugars (e.g., glucose, galactose) in the Agrobacterium infection process?
(A) Enhancement of vir gene induction
(B) Provision of metabolic carbon sources
(C) Suppression of plant immune responses
(D) Binding of VirD2 for nuclear import

2017

**154.** Identify the INCORRECT statement regarding T-DNA processing.
(A) It generates a single-stranded linear DNA molecule (T-strand)
(B) VirD2 cleaves the bottom strand of the T-DNA region at both borders
(C) The synthesis of replacement DNA on the Ti plasmid requires RNA polymerase
(D) The T-strand moves from the right border towards the left border during synthesis

2005

**155.** In particle bombardment (biolistics), the acceleration force most widely used in modern gene guns is provided by:
(A) High-voltage electric discharge
(B) High-pressure helium gas
(C) Gunpowder explosion
(D) Compressed nitrogen gas

NODIA

2025

**156.** The microcarriers onto which DNA is precipitated for biolistics are typically made of:
(A) Gold or tungsten
(B) Silicon or quartz
(C) Aluminum or steel
(D) Platinum or silver

2025

**157.** During a biolistic event, what is the role of the macrocarrier?
(A) Penetration of target plant tissues
(B) Acceleration of the microparticles
(C) Blockage of cell-damaging gas blasts
(D) Precipitation of DNA on microcarriers

(B) Total destruction of RNA molecules
(C) Permanent double helix denaturation
(D) Neutralization of alkaline buffers

2004

**182.** Identify the correct statement regarding chemiluminescent detection in Southern hybridization.
(A) Substrate-to-light conversion
(B) Direct use of radioactive isotopes
(C) Reduction in detection sensitivity
(D) Use of ultraviolet transillumination

2025

**183.** Which of the following is NOT utilized as a method to transfer DNA from a gel to a membrane in Southern blotting?
(A) Capillary transfer
(B) Electrophoretic transfer
(C) Vacuum transfer
(D) Gas-phase bombardment

2025

**184.** PCR-based validation of transformants can sometimes yield false positives. What is a common source of this artifact?
(A) Persisting Agrobacterium residue
(B) Internal chimerism in host plants
(C) Contamination by extracted RNA
(D) Production of T-DNA truncations

2004

**185.** To definitively determine if a transgenic event contains exactly one copy of the transgene via Southern blot, the restriction enzyme chosen for genomic DNA digestion must cut:
(A) Once within the T-DNA region
(B) Once within the vector backbone and once outside
(C) Twice within the T-DNA region
(D) Never within the T-DNA region

2014

**186.** The knowledge of flanking genomic sequences obtained from TAIL-PCR is crucial for:
(A) Generation of initial cDNA libraries
(B) Event-specific marker development
(C) Design of multiple cloning sites
(D) Induction of bacterial competence

2021

**187.** Identify the INCORRECT statement regarding probe labeling for hybridization.
(A) Labeling via random primer extension
(B) Use of biotin or digoxigenin labels
(C) Single-stranded probe hybrid status
(D) Target DNA probe incorporation

2015

**188.** Northern hybridization relies fundamentally on the electrophoretic separation of which molecule?
(A) RNA
(B) Single-stranded DNA
(C) Protein
(D) Double-stranded DNA

2001

**189.** Quantitative real-time RT-PCR (qRT-PCR) measures gene expression by monitoring the:
(A) Exponential phase real-time monitoring
(B) End-point product volume on gels
(C) Ratios of genomic DNA to cDNA
(D) Protein-based chemiluminescent light

2010

**190.** Consider the following steps of a Western blot assay:
I. Incubation with secondary antibody
II. SDS-PAGE
III. Transfer to PVDF membrane
IV. Incubation with primary antibody
Which of the following represents the correct chronological sequence?
(A) $II \rightarrow III \rightarrow I \rightarrow IV$
(B) $III \rightarrow II \rightarrow IV \rightarrow I$
(C) $II \rightarrow III \rightarrow IV \rightarrow I$
(D) $II \rightarrow IV \rightarrow III \rightarrow I$

2017

**191.** In a Western blot, what does the primary antibody specifically bind to?
(A) The PVDF membrane
(B) The blocking agent
(C) The target transgenic protein
(D) The enzyme conjugated to the secondary antibody

2015

**192.** ELISA is an exceptionally useful technique for analyzing transgenic plants because it allows for:
(A) Determination of exact molecular weights
(B) High-throughput quantification
(C) Genomic integration site identification
(D) Spatial protein localization in cells

2002

**193.** Identify the correct objective of immunohistochemical localization.
(A) mRNA quantification in tissue samples
(B) Precise spatial expression mapping
(C) Recombinant enzymatic activity assays
(D) Detection of DNA in whole chromosomes

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Q. Biolistics
R. PEG transformation
S. Plastid transformation

List II: (Requirement/Characteristic)
1. Relies on homologous recombination
2. Avoids tissue culture steps
3. Requires protoplast isolation
4. Uses helium pressure

(A) P-4, Q-3, R-1, S-2
(B) P-1, Q-4, R-2, S-3
(C) P-2, Q-4, R-3, S-1
(D) P-3, Q-1, R-4, S-2

2012

**171.** Transgenes integrated into the chloroplast genome exhibit which highly advantageous trait concerning biosafety?
(A) Maternal inheritance pattern
(B) Direct transmission via pollen grains
(C) Rapid silencing across generations
(D) Position-effect variegation patterns

2012

**172.** Which of the following is NOT true regarding plastid transformation?
(A) Extremely low expression levels
(B) Operon-based multi-gene engineering
(C) Avoidance of transcriptional silencing
(D) Selection-driven homoplasmy status

2006

**173.** What does "homoplasmy" mean in the context of chloroplast transformation?
(A) Transgenes in all plastid genomes
(B) Single transgene copies per genome
(C) Zygosity of specific nuclear traits
(D) Uniformity of the plant phenotype

2015

**174.** The primary benefit of tissue-culture independent protocols (like floral dip) over traditional leaf-disk transformation is:
(A) Avoidance of somaclonal variation
(B) Increased copy number
(C) They work highly efficiently in monocots
(D) Faster production of Agrobacterium cells

2009

**175.** In chloroplast expression cassettes, promoters such as psbA or rrn are utilized because they are:
(A) Derived from standard Ti plasmids
(B) Strong endogenous plastid promoters
(C) Typical eukaryotic nuclear promoters
(D) Activated only during heat shock

2022

**176.** Identify the INCORRECT statement regarding the floral dip method in Arabidopsis thaliana.
(A) It is highly efficient and routinely used
(B) It utilizes a standard Agrobacterium tumefaciens strain
(C) Selection of transformants occurs at the seed germination stage
(D) The resulting seeds are all transgenic

2005

**177.** The primary purpose of performing a Southern hybridization on putative transgenic plants is to determine:
(A) The precise integration pattern and copy number of the transgene
(B) Presence of transgene mRNA species
(C) Determination of protein molecule size
(D) Relative toxicity of transgene events

2019

**178.** Thermal Asymmetric Interlaced (TAIL)-PCR is specifically utilized to:
(A) Exact transgene copy number counts
(B) Isolation of flanking sequences
(C) High GC-content sequence amplification
(D) Validation of internal T-DNA promoters

2005

**179.** In a standard TAIL-PCR procedure, what combination of primers is employed?
(A) Specific nested and degenerate primers
(B) Application of identical primer pairs
(C) Utilization of random hexamer primers
(D) Poly-T and gene-specific primer use

NODIA

2012

**180.** Consider the following essential steps of a Southern Blotting procedure:
I. Transfer to nitrocellulose/nylon membrane
II. Restriction enzyme digestion of genomic DNA
III. Hybridization with a labeled probe
IV. Agarose gel electrophoresis

Which of the following represents the correct chronological sequence?
(A) $II \rightarrow IV \rightarrow I \rightarrow III$
(B) $I \rightarrow II \rightarrow IV \rightarrow III$
(C) $II \rightarrow I \rightarrow IV \rightarrow III$
(D) $IV \rightarrow II \rightarrow I \rightarrow III$

2003

**181.** During the preparation of a gel for Southern transfer, what is the purpose of treating the gel with dilute HCl (depurination)?
(A) Improved large fragment transfer

2007

**158.** Identify the correct mechanism by which Polyethylene Glycol (PEG) facilitates direct gene transfer.
(A) DNA endocytosis via micro-precipitates
(B) Mechanical puncturing of cell walls
(C) Reversible membrane permeability
(D) Opening of pores via electrical fields

2007

**159.** Silicon carbide whisker-mediated transformation achieves DNA delivery by:
(A) DNA transport through cell lipid rafts
(B) Mechanical piercing via agitation
(C) Gas-pressure firing of micro-fibers
(D) Electroporation with conductive fibers

2017

**160.** Given below are two statements: one is labeled as Assertion (A) and the other is labeled as Reason (R).
Assertion (A): Particle bombardment is frequently chosen over Agrobacterium for the transformation of chloroplasts.
Reason (R): Agrobacterium naturally delivers T-DNA to the nucleus, whereas biolistics can physically project DNA directly into plastids.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**161.** A critical disadvantage specifically associated with PEG-mediated transformation is:
(A) Fragile protoplast isolation
(B) Low copy number genomic integration
(C) Efficiency in dicotyledonous plants
(D) Costly gene gun equipment usage

2010

**162.** In a standard helium-driven gene gun (like the PDS-1000/He), what determines the exact pressure at which the gas pulse is released?
(A) The burst rating of the rupture disk
(B) The thickness of the macrocarrier
(C) The size of the microparticles
(D) The vacuum level in the chamber

2008

**163.** The purpose of the stopping screen in a biolistic device is to:
(A) Retention of DNA on microcarriers
(B) Suppression of acoustic shockwaves
(C) Obstruction of the macrocarrier
(D) Filtration of large bacterial cells

2007

**164.** PEG-mediated transformation requires the enzymatic removal of which plant cell structure prior to treatment?
(A) Plasma membrane
(B) Cell wall
(C) Nuclear envelope
(D) Tonoplast

2001

**165.** Identify the INCORRECT parameter for optimization in particle bombardment.
(A) Target distance
(B) Helium pressure
(C) Duration of the electrical pulse
(D) Vacuum level in the bombardment chamber

2014

**166.** The floral dip method of in planta transformation primarily targets which tissues for T-DNA integration?
(A) Collection of mature pollen grains
(B) Unfertilized female germline
(C) Transformation of somatic leaf tissue
(D) Access to the root apical meristem

2010

**167.** Vacuum infiltration improves the efficiency of in planta transformation by:
(A) Denaturation of plant-tissue nucleases
(B) Removal of intercellular air
(C) Suspension temperature augmentation
(D) Puncturing of plant-cell wall units

2023

**168.** In the floral dip protocol, surfactants like Silwet L-77 are added to the inoculation medium to:
(A) Provision of bacterial carbon sources
(B) Induction of the virulence operon
(C) Lethality to non-transformed cells
(D) Reduction of surface tension

2003

**169.** Identify the correct feature regarding chloroplast (plastid) transformation vectors.
(A) Flanking homologous sequences
(B) Reliance on non-homologous end joining
(C) Use of Agrobacterium T-DNA borders
(D) Use of standard CaMV 35S promoters

2023

**170.** Consider the following matching pairs:
List I: (Transformation Technique)
P. Floral dip

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2006

**194.** Which technique would NOT be utilized to confirm the size of a synthesized recombinant protein?
(A) SDS-PAGE
(B) Western Blot
(C) Mass Spectrometry
(D) Northern Blot

2024

**195.** In real-time PCR, SYBR Green I dye provides a fluorescent signal by specifically binding to:
(A) Specific probe sequences
(B) The Taq polymerase enzyme
(C) All double-stranded DNA generated
(D) Single-stranded cDNA

2002

**196.** The accurate quantification of transgene expression by qRT-PCR requires normalization against:
(A) Stable internal reference (housekeeping) genes
(B) Total genomic DNA
(C) Negative control samples
(D) The concentration of the RNA extraction buffer

2011

**197.** A TaqMan probe in qRT-PCR functions via fluorescence resonance energy transfer (FRET) and relies on the 5' to 3' exonuclease activity of:
(A) Reverse transcriptase
(B) Taq polymerase
(C) RNase H
(D) DNA ligase

2017

**198.** Identify the INCORRECT concept regarding Western blot detection.
(A) Simultaneous weight and presence data
(B) Use of blocking buffers like milk
(C) Constant region antibody recognition
(D) Native 3D conformation retention

2003

**199.** The concept of "substantial equivalence" in GMO risk assessment implies that:
(A) Requirement for genetic identicalness
(B) Possession of zero environmental risks
(C) Chemical and nutritional similarity
(D) Equality of DNA and RNA concentrations

2016

**200.** Gene flow from transgenic crops to wild relatives is a significant environmental concern. A proposed biological containment mitigation strategy is:
(A) Chloroplast transgene engineering
(B) Use of strong constitutive promoters
(C) Increased herbicidal agent application
(D) Planting within high-wind environments

2009

**201.** A standard biochemical test utilized to assess the potential allergenicity of a novel transgenic protein is its:
(A) Stability in simulated gastric fluid
(B) Thermal stability in liquid nitrogen
(C) Capability for binding heavy metals
(D) Mobility within agarose gel matrices

2006

**202.** Identify the correct statement for "non-target effects" in Bt crops.
(A) Bt impact on non-target insects
(B) Direct toxicity to host plant tissues
(C) Unintended transfer of genes to weeds
(D) Resistance failure against target pests

NODIA

2004

**203.** According to biosafety containment guidelines, research involving well-characterized agents not known to consistently cause disease in healthy adults is conducted at:
(A) Biosafety Level 1 (BSL-1)
(B) Biosafety Level 4 (BSL-4)
(C) Biosafety Level 3 (BSL-3)
(D) Biosafety Level 2 (BSL-2)

2022

**204.** Which of the following is NOT typically a requirement for Biosafety Level 4 (BSL-4) physical containment?
(A) Use of positive pressure suits
(B) Open-window laboratory benches
(C) Supply air with HEPA filtration
(D) Isolation from general access areas

2016

**205.** Work involving indigenous or exotic agents that may cause serious or potentially lethal disease via inhalation requires containment at:
(A) BSL-1
(B) BSL-3
(C) BSL-1 with HEPA filters
(D) Greenhouses only

2005

**206.** The theoretical use of Genetic Use Restriction Technologies (GURTs), commonly dubbed

"terminator technology", was primarily designed to:
(A) Sterility-based seed protection
(B) Acceleration of natural crop growth
(C) Prevention of gene transfer to bacteria
(D) Termination of viral plant infections

2006

**207.** Why are Cry proteins from B. thuringiensis generally considered to have minimal non-target effects on mammals?
(A) Lack of specific mammalian receptors
(B) Rapid excretion of ingested proteins
(C) Possession of highly alkaline gut pH
(D) Non-consumption of transgenic crops

2004

**208.** Identify the INCORRECT statement regarding biosafety risk assessment.
(A) It proceeds on a case-by-case basis
(B) It requires the demonstration of absolute zero risk for approval
(C) It evaluates both intended and unintended effects of the genetic modification
(D) It considers the biology of the recipient host organism

2021

**209.** Which factor significantly increases the risk of outcrossing (gene flow) from a transgenic plant?
(A) Extensive wind or insect pollination
(B) Exclusive self-pollination mechanisms
(C) Inheritance of functional magic sterility
(D) Implementation of chloroplast changes

2024

**210.** Given below are two statements: one is labeled as Assertion (A) and the other is labeled as Reason (R).
Assertion (A): The Genetic Engineering Appraisal Committee (GEAC) is the apex regulatory body for GMOs in India.
Reason (R): It operates under the Ministry of Agriculture to regulate the commercial release of all agricultural products.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**211.** The Institutional Biosafety Committee (IBSC) is fundamentally constituted at the:
(A) Adoption at the international level
(B) Monitoring at the district level
(C) Organization or institute level
(D) Control at the state government level

2008

**212.** The Review Committee on Genetic Manipulation (RCGM) primarily holds the mandate to:
(A) Issuance of transgenic crop patents
(B) Final commercial release approvals
(C) Research and field trial monitoring
(D) International trade agreement setups

2019

**213.** The GEAC is established under which Ministry of the Government of India?
(A) Ministry of Science and Technology
(B) Ministry of Agriculture and Welfare
(C) MoEFCC (Environment Ministry)
(D) Ministry of Health and Family Welfare

2002

**214.** Identify the correct statement about confined field trials in India.
(A) Mandatory isolation distances
(B) Initiation without regulatory approval
(C) Legal sale of produce in local markets
(D) Monitoring by the GEAC agency solely

2018

**215.** At the district level, which committee is responsible for monitoring the safety regulations of GMOs?
(A) District Level Committee (DLC)
(B) RCGM
(C) State Biotechnology Coordination Committee (SBCC)
(D) IBSC

2018

**216.** Which of the following activities does NOT fall under the direct purview of the GEAC?
(A) Large-scale release of GMO products
(B) Import of genetically modified goods
(C) Low-risk basic laboratory projects
(D) Commercialization of transgenic crops

2014

**217.** The regulatory framework for recombinant DNA safety guidelines in India was initially promulgated under the Environment (Protection) Act of which year?
(A) 1986
(B) 1970
(C) 2001
(D) 1995

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2016

**218.** In the Indian regulatory framework, approval for a transgenic crop is generally granted on a/an:
(A) Event-by-event basis
(B) Species-wide basis
(C) Gene-by-gene basis, ignoring the insertion site
(D) Trait-by-trait basis for all crops simultaneously

2021

**219.** The State Biotechnology Coordination Committee (SBCC) serves to:
(A) State-level biosafety monitoring
(B) GEAC replacement in agricultural zones
(C) Funding of biotechnology research
(D) Patenting of transgenic seed varieties

2004

**220.** Consider the following matching pairs:
List I: (Regulatory Body)
P. IBSC
Q. RCGM
R. GEAC
S. DLC
List II: (Operational Scope)
1. National level commercial approvals
2. Institutional level research monitoring
3. District level compliance monitoring
4. National level confined field trial approvals
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-1, S-3
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-4, R-2, S-3

2018

**221.** Under the Indian Patent Act (1970), an invention must satisfy three core criteria to be patentable. These are novelty, non-obviousness (inventive step), and:
(A) Mandatory environmental safety
(B) Industrial applicability (utility)
(C) Prior international approval
(D) High overall commercial cost

2015

**222.** Section 3(j) of the Indian Patent Act explicitly prohibits the patenting of:
(A) Whole plants, animals, or seeds
(B) Genetically modified microorganisms
(C) Innovative biochemical processes
(D) Novel synthetic DNA sequences

2008

**223.** What is the standard term of a patent granted in India, calculated from the date of filing the application?
(A) 5 years
(B) 10 years
(C) 15 years
(D) 20 years

2021

**224.** Identify the correct statement regarding the patenting of microorganisms.
(A) Genetically modified microorganisms with human intervention and utility are generally patentable
(B) Microorganisms isolated directly from nature without modification are freely patentable in India
(C) The US Supreme Court case Diamond v. Chakrabarty ruled that genetically engineered microbes are non-patentable
(D) The Budapest Treaty bans the patenting of microbes

2010

**225.** The TRIPS agreement, which sets out minimum standards for intellectual property regulation, is administered by the:
(A) WIPO
(B) WTO
(C) WHO
(D) FAO

2002

**226.** Which of the following biotechnological inventions is NOT patentable in India?
(A) A novel vector construct
(B) A traditional method of agriculture or horticulture
(C) A genetically engineered bacterium
(D) A novel process for the extraction of a plant alkaloid

2014

**227.** Under Indian patent law, what mechanism allows the government to permit a third party to produce a patented invention without the consent of the patent owner in cases of public health emergencies?
(A) Compulsory licensing
(B) Benefit sharing
(C) Patent pooling
(D) Substantial equivalence

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# ANSWERS

**1.** (B) Type II
Type II restriction endonucleases are pivotal in molecular biology because they recognize specific palindromic sequences and cleave DNA at precise positions within or near these sites. Unlike other types, they do not require ATP for their catalytic activity, making them reliable and efficient tools for precise DNA manipulation and recombinant DNA technology.

**2.** (A) Isoschizomers
Isoschizomers are pairs of restriction enzymes isolated from different bacterial species that recognize the identical DNA sequence and cleave it in the same manner. While their origin differs, their functional specificity remains the same, providing researchers with alternative enzyme options depending on buffer requirements, reaction temperature, or cost-effectiveness during genomic DNA digestion.

**3.** (C) At distant, non-specific random sites
Type I restriction endonucleases are complex, multi-subunit enzymes that require ATP and S-adenosylmethionine for activity. Although they recognize specific DNA sequences, they cleave the DNA at distant, non-specific random sites, often more than a kilobase away from the recognition site. This characteristic makes them unsuitable for precise gene cloning compared to Type II enzymes.

**4.** (D) Isocaudomers
Isocaudomers are restriction enzymes that recognize different DNA sequences but generate the exact same cohesive or sticky ends upon cleavage. This property allows fragments generated by two different enzymes to be ligated together, although the resulting hybrid site often cannot be recut by either original enzyme, facilitating directional and permanent insertion during cloning.

**5.** (C) They cleave the same recognition sites at different positions
Neoschizomers are a specific subset of isoschizomers that recognize the same DNA sequence but cleave it at different positions within that sequence. This variation results in different types of ends, such as producing a blunt end instead of a sticky end, offering flexibility in experimental design when specific terminal structures are needed for ligation.

**6.** (B) They possess separate, independent R and M enzymes
Type III restriction endonucleases are characterized by their multi-subunit structure, where restriction and methylation activities are part of the same enzyme complex. They recognize non-palindromic sequences and cleave DNA approximately twenty-five base pairs away. They require both ATP and S-adenosylmethionine for full activity, unlike simpler enzymes that function with independent restriction and modification subunits.

**7.** (D) Single polypeptide R-M bifunctional activity
Most Type II restriction endonucleases are homodimers that require divalent magnesium ions and recognize palindromic sequences without needing ATP. However, certain subclasses, such as Type IIG, deviate from this pattern by possessing both restriction and modification activities within a single polypeptide chain. This bifunctional nature allows them to perform dual roles in a simplified structural format.

**8.** (A) Star activity
Star activity refers to the relaxation or alteration of the sequence specificity of restriction endonucleases under non-optimal experimental conditions. Factors such as high glycerol concentration, low ionic strength, or extreme pH can cause an enzyme to cleave sequences similar but not identical to its canonical recognition site, leading to unintended and potentially problematic DNA fragmentation.

**9.** (C) Modified or methylated DNA
Type IV restriction systems are unique because they specifically target and cleave DNA that has undergone modification, such as methylation, hydroxymethylation, or glucosyl-hydroxymethylation. These enzymes serve as a defense mechanism against invading foreign DNA that has been modified to evade other host restriction systems, thereby playing a specialized

NODIA

2024

**240.** Consider the following steps in registering a new variety under the PPV&FR Act:

I. Issue of Certificate of Registration
II. DUS Testing
III. Filing of Application
IV. Advertisement in the Plant Variety Journal for objections

Which of the following represents the correct chronological sequence?

(A) III → IV → II → I
(B) II → III → IV → I
(C) III → II → IV → I
(D) IV → III → II → I

2001

**241.** The Convention on Biological Diversity (CBD) rests on three main pillars. Which of the following is NOT one of them?

(A) The conservation of biological diversity
(B) The elimination of all agricultural biotechnology
(C) The sustainable use of its components
(D) The fair and equitable sharing of benefits arising from genetic resources

2009

**242.** The Nagoya Protocol is a supplementary agreement to the CBD that specifically addresses:

(A) Access and Benefit Sharing (ABS)
(B) Regulation of hazardous waste movement
(C) Global phasing out of chemical pesticides
(D) Intellectual property rights for software

2006

**243.** The Cartagena Protocol primarily regulates:

(A) Safe handling and use of LMOs
(B) Traditional knowledge of indigenous groups
(C) Management of the global Svalbard vault
(D) Implementation of pharmaceutical patents

2013

**244.** Identify the correct procedure established by the Cartagena Protocol for the first intentional transboundary movement of an LMO intended for introduction into the environment.

(A) Substantial Equivalence Evaluation
(B) Advanced Informed Agreement (AIA) procedure
(C) Compulsory Licensing
(D) Open Access Transfer

2013

**245.** Under the Cartagena Protocol, an international platform was established to facilitate the exchange of information on LMOs. This is known as the:

(A) WTO TRIPS Council
(B) Biosafety Clearing-House (BCH)
(C) WIPO Database
(D) National Gene Fund

2016

**246.** Which of the following is explicitly EXCLUDED from the scope of the Advanced Informed Agreement (AIA) procedure under the Cartagena Protocol?

(A) LMOs in transit or intended for contained use
(B) Transgenic seeds intended for field cultivation
(C) Genetically modified fish meant for release into lakes
(D) Transgenic insects for environmental release

2006

**247.** The Nagoya Protocol was formally adopted in which year?

(A) 2010
(B) 1992
(C) 2000
(D) 1970

2025

**248.** A core principle of the Nagoya Protocol is Prior Informed Consent (PIC). This means that researchers must:

(A) Obtain permission to access genetic resources
(B) Informing the public of laboratory trials
(C) Obtaining consumer consent for GMO sales
(D) Securing patents prior to conducting work

2019

**249.** The Convention on Biological Diversity (CBD) was opened for signature at the Earth Summit in Rio de Janeiro in what year?

(A) 1992
(B) 1986
(C) 2001
(D) 1970

2017

**250.** How does the Cartagena Protocol define a Living Modified Organism (LMO)?

(A) Organism with novel genetic combinations
(B) Plants produced via traditional selective breeding
(C) Synthetic chemical compounds utilized in agriculture
(D) Any biological organism that has been patented

*********

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2009

**228.** The patenting of naturally occurring genes is controversial. In major jurisdictions like the US (post-Myriad Genetics ruling), the current stance is that:
(A) Synthetic cDNA created in laboratories is ineligible
(B) Isolated DNA in its natural orientation is patentable
(C) Genomic DNA is ineligible; cDNA is eligible
(D) Strict patenting of all gene sequences is prohibited

2023

**229.** The Budapest Treaty facilitates the patenting of microorganisms by:
(A) Deposit in international authorities
(B) Provision of global patent recognition
(C) Standardization of novelty requirements
(D) Banning of pathogenic microbial usage

2004

**230.** Consider the following steps in a patent application process:
I. Filing of patent application
II. Grant or refusal of patent
III. Examination of the application
IV. Publication of the application
Which of the following represents the correct chronological sequence?
(A) I → IV → III → II
(B) I → III → IV → II
(C) IV → I → III → II
(D) I → II → IV → III

2008

**231.** The International Union for the Protection of New Varieties of Plants (UPOV) Convention aims to:
(A) Establish breeder intellectual property rights
(B) Promotion of open-source sharing models
(C) Banning of transgenic plant cultivation
(D) Regulation of trade in endangered flora

2022

**232.** To be granted protection under plant variety rights (like UPOV or PPV&FR), a new variety must satisfy the DUS criteria, which stand for:
(A) Distinctness, Uniformity, Stability
(B) Diversity, Utility, and Stability levels
(C) Dominance, Utility, and Superiority traits
(D) Distinctness, Usability, and Safety factors

2023

**233.** The Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act was enacted in India in the year:
(A) 2001
(B) 1995
(C) 1970
(D) 1986

2021

**234.** Identify the correct statement concerning Farmers' Rights under the PPV&FR Act, 2001.
(A) Freedom to save and sell farm seed
(B) Sale of branded seeds under breeder names
(C) Strict prohibition of seed saving practices
(D) Royalty payments for unbranded seed trade

2014

**235.** The PPV&FR Act includes "Researchers' Rights", which means researchers can:
(A) Use a protected variety as a source of variation for new breeds
(B) Commercialization without breeder permits
(C) Patenting varieties in foreign countries
(D) Ownership claims after five-year periods

2013

**236.** Which of the following is NOT a legal criterion for registering a plant variety under the PPV&FR Act?
(A) Novelty
(B) Uniformity
(C) Distinctness
(D) Commercial profitability

NODIA

2024

**237.** An "extant variety" under the PPV&FR Act refers to a variety that:
(A) Notified or existing local varieties
(B) Varieties that have reached extinction
(C) Varieties purely genetically engineered
(D) Varieties not yet developed or released

2019

**238.** Benefit sharing under the PPV&FR Act involves:
(A) Community or individual compensation
(B) Sharing of patent rights with the state
(C) Distribution of free seeds to farmers
(D) Sharing of laboratory equipment assets

2015

**239.** The National Gene Fund constituted under the PPV&FR Act is utilized primarily to:
(A) Support for genetic conservation
(B) Funding of government biotech laboratories
(C) Payment of international patent fee costs
(D) Subsidization of chemical fertilizer use

role in maintaining the integrity of the bacterial genome.

**10.** (A) Both A and R are true and R is the correct explanation of A.
Type II restriction enzymes serve as the primary tools in genetic engineering due to their high specificity and predictable cleavage patterns. They identify palindromic DNA sequences and cut precisely at or near those sites. Because they do not require ATP and generate consistent ends, they facilitate the reliable ligation of DNA fragments into vectors, enabling successful gene cloning.

**11.** (D) Strain
The nomenclature for restriction enzymes follows a specific convention where the first letter represents the genus and the next two letters represent the species. The fourth letter, such as the "R" in EcoRI, identifies the specific strain of the organism from which the enzyme was isolated. Roman numerals then indicate the chronological order of discovery in that strain.

**12.** (B) ATP
T4 DNA ligase is an essential enzyme in molecular cloning that catalyzes the formation of a phosphodiester bond between adjacent 3'-hydroxyl and 5'-phosphate termini. To drive this endergonic reaction, the enzyme strictly requires adenosine triphosphate as a cofactor. The ATP is hydrolyzed to provide the energy necessary for the covalent joining of DNA fragments, including blunt and cohesive ends.

**13.** (C) $NAD^+$
While T4 DNA ligase uses ATP, the DNA ligase isolated from Escherichia coli utilizes nicotinamide adenine dinucleotide as its essential cofactor. This enzyme is primarily involved in sealing nicks in double-stranded DNA during replication and repair. In the laboratory, it is less commonly used for general cloning tasks because it cannot efficiently ligate blunt-ended DNA fragments under standard conditions.

**14.** (D) Removal of terminal 5' phosphate groups
Calf Intestinal Phosphatase is frequently employed in cloning to remove the 5'-phosphate groups from linearized plasmid vectors. This modification prevents the vector from self-ligating, as DNA ligase requires a 5'-phosphate to form a phosphodiester bond. By dephosphorylating the vector, researchers significantly increase the efficiency of obtaining recombinant clones containing the desired insert DNA with phosphorylated ends.

**15.** (A) 5' to 3' polymerase and 3' to 5' exonuclease
The Klenow fragment is a large protein fragment produced by the enzymatic cleavage of E. coli DNA Polymerase I. It retains the 5' to 3' polymerase activity and the 3' to 5' proofreading exonuclease activity but lacks the 5' to 3' exonuclease activity. This makes it ideal for tasks like filling in 5' overhangs or labeling DNA ends.

**16.** (B) Simple and permanent heat-inactivation
Shrimp Alkaline Phosphatase offers a significant practical advantage in molecular biology because it can be completely and irreversibly inactivated by heat treatment. Unlike Calf Intestinal Phosphatase, which requires detergent-based extraction or proteinase digestion for removal, SAP is inactivated at sixty-five degrees Celsius. This allows subsequent ligation reactions to proceed in the same tube without risk of dephosphorylating the insert.

**17.** (B) Phosphorylation of 5' hydroxyl termini
T4 Polynucleotide Kinase is a versatile enzyme used to transfer the gamma-phosphate group from ATP to the 5'-hydroxyl terminus of a polynucleotide chain. This reaction is vital for preparing DNA fragments for ligation if they lack 5'-phosphates, such as synthetic oligonucleotides or PCR products. It is also widely used for the end-labeling of DNA or RNA with radioactive phosphorus.

**18.** (C) I and II
The Klenow fragment is generated through the proteolytic processing of DNA Polymerase I and is widely used for filling recessed 5' ends to create blunt ends. While it maintains polymerase and 3' to 5' proofreading activities, it does not possess the 5' to 3' exonuclease activity of the parent enzyme. This specific enzymatic profile prevents the degradation of the template.

**19.** (C) Enzyme-AMP intermediate
The reaction mechanism of DNA ligase involves a three-step process to seal DNA nicks. Initially, the enzyme reacts with a cofactor, either ATP or NAD+, to form a covalent enzyme-adenylate intermediate. This AMP moiety is then transferred to the 5'-phosphate of the DNA

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strand. Finally, the 3'-hydroxyl group attacks the activated phosphate to form the phosphodiester bond, releasing AMP.

**20.** (A) P-2, Q-4, R-3, S-1
Molecular cloning relies on various enzymes with specific roles. T4 DNA ligase seals nicks using ATP, while alkaline phosphatase prevents vector recircularization by removing 5' phosphates. Polynucleotide kinase is utilized for 5' end labeling or phosphorylation. The Klenow fragment is essential for filling in recessed 5' sticky ends to produce blunt-ended DNA, facilitating a variety of downstream recombinant DNA applications.

**21.** (C) T4 DNA polymerase
T4 DNA polymerase is the enzyme of choice for blunt-ending DNA fragments that possess 3' overhangs. It utilizes its potent 3' to 5' exonuclease activity to chew back the protruding single-stranded 3' ends until they meet the double-stranded region. In the presence of dNTPs, it can also fill in recessed 3' ends, making it a highly versatile tool.

**22.** (B) Gamma-phosphate exchange at 5' ends
In the presence of excess ADP, T4 Polynucleotide Kinase can catalyze an exchange reaction at the 5' end of DNA. The enzyme first removes the existing 5'-phosphate group and transfers it to ADP to form ATP. Subsequently, it re-phosphorylates the 5'-hydroxyl group using a phosphate from the ATP pool. This method is often used to label DNA with radioactive phosphorus.

**23.** (D) Ampicillin and Tetracycline
The pBR322 plasmid is a classic cloning vector that features two distinct selectable markers: the ampicillin resistance gene and the tetracycline resistance gene. These markers allow researchers to identify host cells that have taken up the plasmid. Furthermore, the presence of unique restriction sites within these genes enables the use of insertional inactivation to screen for recombinant plasmids containing foreign DNA.

**24.** (A) MCS located within the lacZ' gene
The pUC19 vector is designed for high-efficiency cloning and features a multiple cloning site located within the lacZ' gene. This arrangement allows for blue-white screening, where the insertion of foreign DNA disrupts the production of the α-peptide of β-galactosidase. Consequently, recombinant colonies remain white on media containing X-gal, while non-recombinant colonies turn blue, simplifying the identification of successful clones.

**25.** (C) ColE1
The origin of replication found in the pBR322 plasmid is derived from the naturally occurring ColE1 plasmid of Escherichia coli. This origin determines the plasmid's copy number and its compatibility with other plasmids. ColE1-type origins are characterized by a relaxed replication control mechanism, which allows the plasmid to be maintained at moderate to high copy numbers within the bacterial host.

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**26.** (A) Plasmids with different replication origins are typically incompatible
Plasmid incompatibility occurs when two plasmids cannot be stably maintained in the same cell line because they share identical replication or partition systems. Plasmids with different origins are typically compatible and can co-exist. However, if they utilize the same regulatory molecules to control their copy number, they will compete, eventually leading to the loss of one plasmid from the daughter cells.

**27.** (D) RNA I
In the ColE1 replication system, RNA I acts as a small antisense RNA molecule that regulates the plasmid's copy number. It binds to the pre-primer RNA II, preventing it from forming the stable DNA-RNA hybrid required for the initiation of replication by DNA polymerase I. This antisense interaction ensures that the plasmid concentration remains within a specific range inside the host.

**28.** (B) Rop gene
The pUC19 vector achieves a significantly higher copy number than pBR322 because it lacks the rop gene. The Rop protein normally stabilizes the interaction between the regulatory RNAs, RNA I and RNA II, leading to more effective inhibition of replication initiation. Without this protein, the inhibitory effect is weakened, allowing the plasmid to replicate more frequently and reach higher concentrations.

**29.** (C) 3' T overhangs
The pGEM-T vector system is specifically engineered for the efficient cloning of PCR products generated by Taq polymerase. These

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vectors are linearized and possess a single 3' terminal thymidine overhang at both ends. This design exploits the fact that Taq polymerase adds a non-templated deoxyadenosine to the 3' ends of PCR fragments, allowing for easy and direct cohesive-end ligation.

**30.** (C) I and III
Plasmid pBR322 was one of the first widely used artificial vectors, constructed by combining segments from several natural plasmids. While cloning into the BamHI site disrupts tetracycline resistance, not ampicillin, pUC19 improved upon this by incorporating a multiple cloning site within the lacZ' gene. This allows researchers to use blue-white screening as a convenient method for identifying recombinant colonies during experiments.

**31.** (A) Incompatibility
If two plasmids utilize the same repressor control system for regulating their replication, they belong to the same incompatibility group. Because the replication machinery cannot distinguish between the two plasmids, it will treat them as a single population. Over successive rounds of cell division, one plasmid will inevitably be lost, as the cell cannot maintain both due to the shared regulation.

**32.** (B) They exist as separate structural units
In the pUC19 vector, the origin of replication and the ampicillin resistance gene are situated as distinct, separate functional units. The ori region is responsible for autonomous replication and copy number control, while the bla gene provides the selectable marker. This modular organization is typical of modern cloning vectors, ensuring that the essential functions of replication and selection do not interfere.

**33.** (B) A-complementation region
The pBR322 vector is characterized by its origin of replication, specific selectable markers like ampicillin and tetracycline resistance, and several unique restriction sites. However, it does not contain the α-complementation region associated with the lacZ' gene. This feature was introduced later in the pUC series of vectors to enable the more convenient blue-white screening method for identifying recombinant clones.

**34.** (A) 52 kb
The wild-type bacteriophage Lambda has a genome of approximately forty-nine kilobases, and its protein head has a strict physical limit for DNA packaging. It can accommodate DNA molecules that are between seventy-eight and one hundred and five percent of the wild-type genome size. This translates to a maximum capacity of roughly fifty-two kilobases, beyond which the phage particles cannot be assembled.

**35.** (C) Replacement vectors contain a stuffer fragment that is discarded during cloning
Replacement vectors in the Lambda phage system are designed to carry larger DNA fragments than insertion vectors. They contain a central "stuffer" fragment that is not essential for the lytic cycle. During the cloning process, this stuffer is removed by restriction enzyme digestion and replaced with foreign DNA. This mechanism allows the vector to accommodate inserts ranging from nine to twenty-three kilobases.

**36.** (D) cI (repressor)
The lambda gt10 vector is a popular insertion vector used for cDNA cloning. It contains a unique EcoRI restriction site located within the cI repressor gene. When a foreign DNA fragment is inserted at this site, the cI gene is inactivated. This allows for the selection of recombinants, as they form clear plaques on specific E. coli strains, unlike non-recombinants.

**37.** (C) 9-23 kb
Lambda EMBL4 is a high-capacity replacement vector frequently used for the construction of genomic libraries. By removing the non-essential stuffer fragment, the vector can accommodate large inserts of foreign DNA, typically ranging from nine to twenty-three kilobases. This large capacity makes it much more efficient than plasmid vectors for representing the entire genome of an organism in a library.

**38.** (B) Single-stranded circular
M13 is a filamentous bacteriophage that infects Escherichia coli. A unique feature of this system is that while the phage replicates inside the host as a double-stranded circular DNA molecule, it is packaged and secreted into the medium as single-stranded circular DNA. This property is highly valuable for applications requiring single-

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**96.** (B) Aequorea victoria
The Green Fluorescent Protein was originally isolated from the bioluminescent jellyfish Aequorea victoria. It is a highly valuable reporter because it fluoresces spontaneously when exposed to blue or ultraviolet light, without requiring any external substrates or cofactors. This property allows for the real-time, non-invasive imaging of protein localization and dynamic processes within living cells across many different organisms in biological research.

**97.** (B) Luciferin, ATP, and $O_2$
The light-emitting reaction catalyzed by the enzyme luciferase is widely used in highly sensitive quantitative assays. To produce bioluminescence, the enzyme requires the substrate luciferin, along with adenosine triphosphate and molecular oxygen. The intensity of the light produced is proportional to the amount of luciferase present, making it an excellent tool for measuring promoter activity and gene expression levels in various biological systems.

**98.** (D) The lethal gene is activated specifically by the insertion of foreign DNA
Positive selection systems are designed to allow only the growth of recombinant colonies, eliminating the need for extensive screening. This is usually achieved by the insertional inactivation of a lethal gene, such as ccdB or sacB. If no DNA is inserted, the lethal gene remains active and kills the host cell. Therefore, the lethal gene is inactivated, not activated, by the insertion of foreign DNA.

**99.** (C) Induction of the lac promoter
IPTG is a synthetic molecule used in molecular biology to induce the expression of genes under the control of the lac promoter. It acts by binding to the lac repressor and causing it to dissociate from the operator. Unlike the natural inducer allolactose, IPTG is not broken down by β-galactosidase, ensuring a constant and stable level of induction throughout the growth of the culture.

**100.** (D) I, II, III, IV, and V
Selectable marker genes are essential for identifying transformed cells. Common markers include nptII for kanamycin resistance, hpt for hygromycin, bar for phosphinothricin, and pat for bialaphos. Additionally, the cat gene provides resistance to chloramphenicol. These genes encode enzymes that detoxify the corresponding antibiotics or herbicides, allowing only the transgenic cells to survive and proliferate on selective media during the transformation process.

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**101.** (C) Phosphorylation of aminoglycosides
The nptII gene, which encodes neomycin phosphotransferase II, is a standard selectable marker in plant and bacterial transformation. It provides resistance to aminoglycoside antibiotics like kanamycin and neomycin. The enzyme works by catalyzing the phosphorylation of these antibiotics, which prevents them from binding to the ribosomal subunits of the host. This effectively neutralizes their toxic effects on protein synthesis, allowing transgenic cells to grow.

**102.** (B) Aminoglycoside phosphorylation
The hpt gene encodes the enzyme hygromycin B phosphotransferase, which provides resistance to the antibiotic hygromycin B. Like neomycin phosphotransferase, this enzyme functions by phosphorylating the antibiotic molecule. The addition of a phosphate group prevents hygromycin B from interacting with the ribosome and inhibiting protein synthesis. This marker is particularly useful in plant transformation because hygromycin B is highly effective at killing non-transgenic plant cells.

**103.** (D) Phosphinothricin (glufosinate)
The bar gene is a widely used selectable marker and herbicide resistance trait in plant biotechnology. It encodes the enzyme phosphinothricin acetyltransferase, which detoxifies herbicides containing phosphinothricin or glufosinate. The enzyme acetylates the herbicide, rendering it inactive. This allows the transgenic plants to survive applications of glufosinate, which would otherwise be lethal by inhibiting the essential plant enzyme glutamine synthetase.

**104.** (A) Inhibition of glutamine synthetase
Phosphinothricin, the active ingredient in several broad-spectrum herbicides, is a structural analog of glutamate. It acts by competitively inhibiting the enzyme glutamine synthetase in plants. This inhibition leads to a rapid accumulation of toxic ammonia and a deficiency in the amino acid glutamine, ultimately causing the death of the plant. Herbicide-resistant crops use genes like bar to detoxify this compound before it can act.

F-factor plasmid of E. coli. The F-factor's robust replication and partition systems ensure that the BAC is maintained stably at a single copy per cell. This low copy number minimizes deleterious recombination events and allows for the stable maintenance of large inserts.

**49.** (B) Bacteriophage P1
P1-derived Artificial Chromosomes are large-capacity vectors that utilize the replication machinery of the P1 bacteriophage. They incorporate elements from the P1 system, such as the Cre-loxP site-specific recombination system and the P1 plasmid origin of replication. PACs can accommodate inserts up to three hundred kilobases and are maintained at a low copy number to ensure the stability of the cloned DNA.

**50.** (B) I, II, III, IV
In molecular cloning, different vectors are chosen based on the size of the DNA to be inserted. Standard plasmids like pUC19 have the smallest capacity, followed by cosmids which can hold up to forty-five kilobases. BACs offer significantly larger capacity, reaching three hundred kilobases, while YACs provide the highest capacity, often exceeding five hundred kilobases for very large-scale genomic projects.

**51.** (C) Stability without chimeric artifacts
While Yeast Artificial Chromosomes are capable of carrying very large DNA inserts, they are frequently prone to structural instability and the formation of chimeric artifacts. Chimerism occurs when two unrelated DNA fragments are ligated together into a single YAC, complicating the assembly of genomic maps. Despite these drawbacks, YACs remain valuable for cloning sequences that are too large for bacterial systems.

**52.** (A) Electroporation
Because Bacterial Artificial Chromosomes are very large DNA molecules, they are fragile and difficult to introduce into host cells using standard chemical methods. Electroporation is the preferred technique, as it uses brief, high-voltage pulses to create transient pores in the bacterial membrane. This allows the large BAC DNA to enter the cell with higher efficiency and less risk of mechanical shearing.

**53.** (C) BAC
To stably clone a contiguous genomic sequence of one hundred and twenty kilobases in E. coli, a Bacterial Artificial Chromosome is the most appropriate vector. Plasmids and Lambda vectors lack the necessary capacity, and cosmids are limited to about forty-five kilobases. BACs, being derived from the F-factor, can easily accommodate this size while maintaining high stability at a low copy number.

**54.** (D) cos site
A Yeast Artificial Chromosome vector includes essential elements for maintenance in yeast, such as URA3 as a selectable marker, TEL sequences for end stability, and the CEN4 centromere for segregation. However, it does not typically contain a cos site. The cos site is a specific feature of Lambda phage-based vectors, such as cosmids, where it is required for DNA packaging.

**55.** (C) A cos site
The defining feature of a cosmid vector is the presence of the cos site, which is derived from the bacteriophage Lambda. This specific sequence is recognized by the phage packaging machinery, allowing the circular cosmid DNA to be linearized and packaged into viral heads in vitro. Without the cos site, the vector would behave simply as a standard plasmid and cannot be packaged.

**56.** (B) trp and lac promoters
The tac promoter is a powerful hybrid promoter used for the high-level expression of recombinant proteins in Escherichia coli. It was created by fusing the -35 region of the tryptophan operon promoter with the -10 region of the lactose operon promoter. This combination results in a promoter that is stronger than either of its parents while remaining inducible by IPTG.

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**57.** (B) T7 RNA polymerase
The T7 expression system is highly efficient because it utilizes the T7 RNA polymerase, which is extremely specific for its own promoter. For this system to function, the host E. coli strain must be engineered to express the T7 RNA polymerase, often under the control of an inducible promoter like lac. This allows for the precise and massive production of target mRNA.

which facilitates the assembly of the VirD1/ VirD2 complex at the border. This interaction significantly enhances the efficiency of T-DNA nicking and subsequent transfer, particularly in strains where the natural transfer rate is low.

**78.** (A) Shielding of electrostatic repulsion
Preparing chemically competent E. coli with calcium chloride involves treating the cells with divalent cations at low temperatures. These positively charged ions help to shield the electrostatic repulsion between the negatively charged DNA molecules and the negatively charged bacterial lipopolysaccharides. This stabilization allows the DNA to adhere to the cell surface, making it ready for uptake during the subsequent heat shock step.

**79.** (C) Thermal gradient-driven DNA uptake
The brief heat shock step during chemical transformation is thought to create a thermal gradient across the bacterial cell membrane. This temperature shift induces the uptake of DNA that has been stabilized on the cell surface by calcium ions. Additionally, the heat may cause transient changes in membrane fluidity or pore formation, allowing the plasmid DNA to enter the cytoplasm efficiently.

**80.** (A) I and III
Electroporation is a highly efficient method for introducing DNA into cells using short, high-voltage electrical pulses. These pulses create transient pores in the plasma membrane, through which DNA can enter. Unlike chemical transformation, electroporation requires a low-conductivity buffer to prevent arcing. High salt concentrations must be strictly avoided, as they can cause electrical discharge and lead to significant cell death and sample loss.

**81.** (A) Higher transformation efficiency
The use of rubidium chloride in place of or alongside calcium chloride is part of more advanced protocols for preparing competent cells. This method often yields significantly higher transformation efficiencies compared to the standard calcium chloride technique. It is particularly useful when working with large plasmids or when a high number of transformants is required for the construction of comprehensive cDNA or genomic libraries.

**82.** (C) Use of low-salt or glycerol washes
For successful electroporation, it is essential to use buffers with very low ionic strength. This is typically achieved by washing the bacterial cells multiple times with ice-cold deionized water or a ten percent glycerol solution to remove all traces of salts from the growth medium. Low conductivity prevents electrical arcing, which would otherwise destroy the cells and the DNA during the high-voltage pulse.

**83.** (D) Resistance and capacitance
In an electroporation experiment, the time constant is a critical parameter that represents the duration of the electrical pulse. It is mathematically defined as the product of the resistance and the capacitance of the electrical circuit. Adjusting these values allows researchers to optimize the delivery of DNA into specific cell types while minimizing the lethal effects of the high-voltage discharge on the population.

**84.** (A) Arcing
Arcing is a violent electrical discharge that occurs during electroporation if the sample's conductivity is too high. This phenomenon is usually caused by residual salts in the cell suspension or DNA preparation. Arcing generates localized heat and pressure that kill the bacteria and degrade the DNA, resulting in a total failure of the transformation and necessitating a thorough cleaning of the electroporation chamber.

**85.** (B) 12-18 kV/cm
The field strength required for the efficient electroporation of E. coli is typically quite high, ranging from twelve to eighteen kilovolts per centimeter. This is achieved by applying a high voltage across a very narrow gap in a specialized cuvette. This intense electrical field is necessary to overcome the barrier of the bacterial cell wall and membrane, facilitating the entry of recombinant DNA molecules.

**86.** (C) Rapidly warming the cells to $37^{\circ}C$ before adding DNA
The preparation of chemically competent E. coli requires meticulous care. Cells must be harvested during their mid-logarithmic growth phase and maintained strictly on ice throughout the washing and salt-treatment steps. Rapidly warming the cells before the addition of DNA is incorrect and would significantly reduce their

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and processing of the T-DNA by the VirD1/ VirD2 endonuclease complex. The Right border is particularly critical, as it acts as the initiation point for T-strand synthesis.

**68.** (B) The vir genes and T-DNA are located on separate plasmids
In the Agrobacterium binary vector system, the vir genes and the T-DNA are located on two separate plasmids. The binary vector carries the T-DNA and an origin for E. coli and Agrobacterium, while the helper Ti plasmid provides the vir functions in trans. This system is more flexible and easier to manipulate than the co-integrate system, which requires a recombination event.

**69.** (C) Binary vector
pBIN19 is a widely used first-generation binary vector for Agrobacterium-mediated plant transformation. It contains the T-DNA borders, a multiple cloning site, and selectable markers for both bacteria and plants. Because it possesses a broad-host-range origin of replication, it can be easily shuttled between E. coli and Agrobacterium, where the host's helper Ti plasmid provides the necessary virulence proteins for T-DNA transfer.

**70.** (A) P-3, Q-2, R-1, S-4
The Agrobacterium infection process involves specialized Vir proteins with distinct roles. VirA acts as a sensor kinase for plant phenolics, while VirD2 is an endonuclease that cleaves T-DNA borders. VirE2 is a single-stranded DNA binding protein that protects the T-strand during transport. Finally, the VirB operon encodes the components of the Type IV secretion system, which facilitates the export of the T-complex.

**71.** (D) Covalent 5' attachment of VirD2
During T-DNA processing, the VirD2 protein plays a crucial dual role. It first introduces a site-specific nick at the right border and then remains covalently attached to the 5' end of the resulting single-stranded T-strand. This attachment protects the DNA from exonuclease degradation and contains nuclear localization signals that guide the T-complex into the plant nucleus once inside the host cell.

**72.** (A) A helper plasmid named pSoup
The pGreen series of binary vectors is characterized by its small size and versatility. However, it lacks a functional origin of replication for Agrobacterium on its own. It relies on a specialized helper plasmid called pSoup, which provides the necessary RepA protein in trans. This dual-plasmid requirement ensures that the pGreen vector remains stable and can replicate within the Agrobacterium host.

**73.** (D) vir gene operon
In engineered binary vectors, the T-DNA region is modified to include only the gene of interest and essential selectable markers. The vir gene operon is always excluded from the T-DNA and remains on the helper plasmid. This is because the vir genes are only needed within the Agrobacterium cell to facilitate the transfer process and are not required or desired in the plant.

**74.** (A) Acetosyringone
Acetosyringone is a natural phenolic compound released by plant cells upon wounding. It serves as a potent signaling molecule that is sensed by the VirA protein of Agrobacterium tumefaciens. This detection triggers a phosphorylation cascade that activates the expression of the entire vir operon, initiating the process of T-DNA processing and transfer into the host plant cell.

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**75.** (C) vir genes but no T-DNA borders
In a binary vector system, the helper Ti plasmid is "disarmed," meaning its T-DNA region has been removed. It retains the complete set of vir genes, which are essential for processing and transferring the T-DNA from the separate binary vector. This separation of the vir functions and the T-DNA makes the system safer and easier to use for genetic engineering in plants.

**76.** (B) It lacks the essential vir genes for transfer
A "disarmed" Ti plasmid is one where the oncogenes within the T-DNA region have been deleted to prevent the formation of crown gall tumors. Crucially, it still maintains all the essential vir genes required for the transfer process. This modification allows the bacterium to act as a delivery vehicle for foreign genes without causing disease in the recipient plant tissue.

**77.** (D) Enhance the efficiency of T-DNA transfer
The "overdrive" sequence is a twenty-four base pair enhancer element located near the right border of certain Ti plasmids. It serves as a binding site for the VirC1 protein,

**58.** (A) SD for prokaryotes; Kozak for eukaryotes
The Shine-Dalgarno and Kozak sequences are critical for translation initiation but function in different domains of life. The Shine-Dalgarno sequence is found in prokaryotic mRNAs and guides the 30S ribosomal subunit to the start codon. In contrast, the Kozak sequence is found in eukaryotic mRNAs and facilitates the recognition of the initiation codon by the 40S ribosomal subunit during scanning.

**59.** (C) Glutathione-Sepharose beads
The Glutathione S-transferase tag is a widely used fusion partner that facilitates the purification of recombinant proteins. GST has a high and specific affinity for glutathione. By passing a cell lysate over a column of Glutathione-Sepharose beads, the GST-tagged protein binds tightly while other proteins are washed away. The target protein is then eluted using a buffer containing reduced glutathione.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Hexahistidine tags are popular for protein purification because they consist of six consecutive histidine residues that bind strongly to transition metal ions. In Immobilized Metal Affinity Chromatography, ions like nickel or cobalt are tethered to a solid matrix. When the tagged protein passes through the column, it is selectively retained. Elution is typically achieved by adding imidazole, which competes for the binding sites.

**61.** (B) It requires nickel affinity chromatography for purification
Maltose Binding Protein is a versatile fusion tag that is primarily known for its ability to significantly increase the solubility of difficult-to-express recombinant proteins in E. coli. Purification is achieved through its high affinity for amylose resin, not nickel. After binding and washing, the fusion protein is eluted with maltose. MBP can later be removed using specific proteases if needed.

**62.** (A) Constitutive plant promoter
The Cauliflower Mosaic Virus 35S promoter is one of the most widely used constitutive promoters in plant biotechnology. It is highly active in nearly all tissues and at almost all stages of plant development in a broad range of species. This makes it an ideal choice for driving the high-level expression of transgenes, such as those conferring antibiotic resistance or insect tolerance.

**63.** (C) Shine-Dalgarno sequence
When constructing an expression cassette for a eukaryotic host, several elements are essential, including enhancers, a polyadenylation signal for mRNA stability, and the Kozak sequence for translation initiation. However, the Shine-Dalgarno sequence is a prokaryotic feature and is not recognized by eukaryotic ribosomes. Therefore, it is generally unnecessary and irrelevant for the proper functioning of a transgene in eukaryotic cells.

**64.** (D) 16S rRNA of the 30S subunit
In prokaryotes, translation initiation begins when the Shine-Dalgarno sequence in the mRNA base-pairs with a complementary sequence at the 3' end of the 16S ribosomal RNA. This interaction correctly positions the 30S ribosomal subunit over the AUG start codon. This precise alignment is crucial for the efficient and accurate translation of the genetic code into a functional polypeptide chain.

**65.** (B) Allolactose
The lactose operon is regulated by the lac repressor, which binds to the operator and blocks transcription. In nature, allolactose, an isomer of lactose, acts as the natural inducer by binding to the repressor and causing it to release from the DNA. In the laboratory, the synthetic analog IPTG is more commonly used because it is not metabolized by the bacteria.

**66.** (B) A protease cleavage site
Recombinant proteins are often expressed with fusion tags to aid in purification or solubility. To recover the native target protein, a specific protease cleavage site is engineered between the tag and the gene of interest. After the fusion protein is purified, it is treated with a specific protease, such as Thrombin or Factor Xa, which recognizes and cuts only at that site.

**67.** (D) 25 bp imperfect direct repeats (Right and Left borders)
The T-DNA region of the Agrobacterium Ti plasmid is flanked by two twenty-five base pair sequences known as the Right and Left borders. These sequences are imperfect direct repeats that are essential for the recognition

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competence. The heat shock should only be applied briefly after the DNA has had time to bind.

**87.** (C) Highly permeable growing membranes
Bacteria are harvested during the logarithmic growth phase for competent cell preparation because their cell membranes and walls are most dynamic and permeable. In this rapidly dividing state, the cells are more susceptible to the chemical and physical treatments that induce competence. Using cells from the stationary phase results in much lower transformation efficiencies due to their more rigid and less receptive cell envelopes.

**88.** (A) Elevated sample salt concentration
Arcing during electroporation is most frequently caused by an elevated salt concentration in the sample. High ionic strength increases the conductivity of the medium, allowing the electrical current to flow too easily and causing a spark or arc. To prevent this, both the bacterial cells and the DNA must be thoroughly desalted or resuspended in a non-conductive medium like water or glycerol.

**89.** (B) Insertional inactivation
Insertional inactivation is a screening technique where the insertion of a foreign DNA fragment into a selectable marker gene disrupts its function. In pBR322, cloning into the SalI site within the tetracycline resistance gene makes the host cell sensitive to that antibiotic. By comparing growth on different media, researchers can identify recombinant colonies that are resistant to ampicillin but sensitive to tetracycline.

**90.** (B) I, III and IV
Blue-white screening is a powerful tool for identifying recombinant clones. It utilizes a host strain with a deleted lacZ α sequence and a vector that provides this sequence. If the multiple cloning site within the lacZ' gene is interrupted by an insert, no functional β-galactosidase is formed. Consequently, these recombinant colonies appear white on X-gal media, while non-recombinants produce active enzyme and turn blue.

**91.** (C) Poisoning of host DNA gyrase
The ccdB gene is used in positive selection vectors to ensure that only recombinant cells survive. The CcdB protein is a potent toxin that targets and poisons host DNA gyrase, leading to cell death. When a foreign DNA fragment is successfully inserted into the ccdB gene, the toxin's production is disrupted. This allows only the bacteria containing the recombinant plasmid to grow and form colonies.

**92.** (C) Peptide complementation of lacZ
A-complementation is the basis for blue-white screening. It involves the interaction between two inactive fragments of the β-galactosidase enzyme: the α-peptide, encoded by the vector, and the omega-peptide, encoded by the host. When both are present, they assemble into a functional enzyme. Insertion of foreign DNA into the vector's α-peptide gene prevents this complementation, allowing for the easy visual identification of recombinants.

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**93.** (C) Chromogenic lactose analog
X-gal is a chromogenic substrate used in molecular biology as an analog of lactose. It is cleaved by the enzyme β-galactosidase to produce galactose and an indigo-colored precipitate. In blue-white screening, the presence of this blue color indicates that the enzyme is functional and the vector is non-recombinant. White colonies indicate that the enzyme has been inactivated by the insertion of foreign DNA.

**94.** (D) EcoRI
Reporter genes are used to monitor gene expression and protein localization. Common examples include uidA, which produces a blue color, gfp, which fluoresces green, and luc, which produces light. These genes provide easily measurable signals. In contrast, EcoRI is a restriction endonuclease used for cutting DNA and does not function as a reporter for gene expression or cellular activity in recombinant technology.

**95.** (C) X-Gluc
The GUS reporter system utilizes the uidA gene from E. coli, which encodes the enzyme β-glucuronidase. To detect its expression in transgenic plants, tissues are incubated with the substrate X-Gluc. The enzyme cleaves X-Gluc to form an intermediate that oxidizes into a stable, insoluble blue precipitate. This allows researchers to visualize the precise spatial and temporal patterns of gene expression throughout the plant.

stranded templates, such as Sanger sequencing or site-directed mutagenesis.

**39.** (D) Infection with a helper phage
Phagemids are hybrid vectors that contain both a plasmid origin of replication and a filamentous phage origin, such as M13. To package the single-stranded phagemid DNA into viral particles, the host cell must be co-infected with a helper phage. The helper phage provides all the necessary structural proteins and enzymatic machinery that the phagemid itself lacks, facilitating the secretion of virions.

**40.** (B) I, III and IV
Phagemids like pBluescript are versatile vectors that combine the benefits of plasmids and phages. They possess two origins of replication, allowing them to be maintained as double-stranded plasmids or packaged as single-stranded DNA. Crucially, they do not encode the structural proteins for phage assembly, requiring a helper phage for virion production. These features make them indispensable tools in modern molecular biology.

**41.** (C) Host cell lysis and subsequent death
M13 bacteriophage is unusual because it does not cause host cell lysis or death. Instead, it establishes a chronic infection where the virus is continuously extruded through the cell envelope as filamentous particles while the host continues to grow and divide, albeit at a slower rate. This allows for the convenient collection of viral DNA from the surrounding culture medium over time.

**42.** (A) T3 and T7 promoters
The pBluescript phagemid is designed with two different bacteriophage promoters, typically T3 and T7, flanking the multiple cloning site. This arrangement allows for the efficient in vitro transcription of either strand of the inserted DNA using the corresponding RNA polymerase. This is particularly useful for generating high-quality RNA probes or for synthesizing large amounts of RNA for functional studies and experiments.

**43.** (D) Functional replication origins
Commercial in vitro packaging extracts for Lambda phage are prepared from E. coli cells infected with defective prophages. These extracts provide all the essential structural components, such as empty phage heads, tails, and the packaging enzymes required to assemble infectious virions. However, they do not contain functional replication origins, as their purpose is solely the physical packaging of pre-existing DNA molecules.

**44.** (B) cos site
The Lambda phage genome is a linear double-stranded DNA molecule that possesses short, single-stranded complementary overhangs at its ends. These regions, known as cos sites, allow the DNA to circularize through base-pairing once it enters the Escherichia coli host cell. This circularization is a critical step for both the replication of the viral genome and the initiation of the lysogenic cycle.

**45.** (C) 35-45 kb
Cosmids are hybrid vectors that combine the high-copy replication of plasmids with the packaging system of Lambda phage. By containing the cos site, they can be packaged into phage heads in vitro. Because they lack most viral genes, they can accommodate very large DNA inserts, typically ranging from thirty-five to forty-five kilobases, making them ideal for genomic library construction.

**46.** (D) CEN, TEL, and ARS components
Yeast Artificial Chromosomes are designed to replicate and behave like natural chromosomes within yeast cells. To achieve this, they must include three essential structural elements: an Autonomously Replicating Sequence for the initiation of replication, a Centromere for proper segregation during cell division, and Telomeres at the ends to ensure stability. These components allow YACs to carry extremely large fragments of DNA.

**47.** (A) Stringent copy number regulation
Fosmids are specialized cosmid vectors based on the bacterial F-factor, which ensures they are maintained at a very low copy number, usually just one or two per cell. This stringent regulation is a major advantage because it significantly increases the stability of large genomic inserts, particularly those containing sequences that might be toxic or unstable if present at higher copy numbers.

**48.** (B) F-factor plasmids
Bacterial Artificial Chromosomes are widely used for cloning very large DNA fragments. They are derived from the naturally occurring

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**105.** (B) Metabolic utilization of mannose
Phosphomannose isomerase selection is a positive metabolic selection system that differs from traditional antibiotic-based methods. It allows plants to use mannose as a carbon source by converting mannose-6-phosphate into fructose-6-phosphate. Non-transgenic cells cannot perform this conversion and suffer from growth inhibition due to the accumulation of mannose-6-phosphate. This system provides a non-toxic alternative for selecting transformants, avoiding concerns associated with antibiotic resistance genes.

**106.** (D) Neomycin phosphotransferase (nptII)
Negative selectable markers, such as codA, sacB, or diphtheria toxin A, are genes that produce a product lethal to the cell expressing them. They are often used to select against the presence of specific DNA sequences. In contrast, nptII is a positive selectable marker. It provides a survival advantage by conferring resistance to kanamycin, allowing only the cells that possess the gene to grow.

**107.** (D) Protein translation
Hygromycin B is an aminoglycoside antibiotic that is highly toxic to both prokaryotic and eukaryotic cells. Its primary mechanism of action is the interference with the translocation step of protein translation on the ribosome. By causing translational errors and inhibiting the synthesis of new proteins, it effectively stops cell growth and leads to cell death in sensitive plant tissues that lack the protective hpt gene.

**108.** (A) Stimulation without toxic agents
The use of the pmi gene is classified as positive metabolic selection because it grants the transgenic cells a unique ability to utilize a specific nutrient, such as mannose. Unlike traditional selection, which relies on the lethal action of antibiotics or herbicides to kill non-transformed cells, this system promotes the growth of transformants on a medium where the nutrient source is otherwise unusable.

**109.** (C) The 30S ribosomal subunit
Kanamycin is a potent aminoglycoside antibiotic that exerts its effect by specifically binding to the 30S subunit of the bacterial ribosome. This binding interferes with the initiation of protein synthesis and causes the misreading of mRNA, leading to the production of non-functional or toxic proteins. In plant biotechnology, kanamycin also affects the ribosomes within chloroplasts and mitochondria, which are structurally similar to bacterial ribosomes.

**110.** (A) Both A and R are true and R is the correct explanation of A.
PMI-based selection is increasingly preferred in crop biotechnology because it offers a non-antibiotic alternative for identifying transgenic plants. This addresses public and regulatory concerns regarding the potential horizontal transfer of antibiotic resistance genes to environmental bacteria. By utilizing a simple metabolic enzyme, researchers can achieve high transformation efficiency while improving the overall biosafety profile of the genetically modified crop for commercial release.

**111.** (D) It ensures the insert is integrated in the correct orientation
Directional cloning is a superior technique that uses two different restriction enzymes to create unique, non-compatible sticky ends on both the vector and the insert. This ensures that the DNA fragment can only be ligated into the vector in one specific orientation. This is crucial for expression studies where the gene must be correctly aligned with the promoter to be properly transcribed and translated.

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**112.** (B) Taq DNA polymerase
TA cloning is a simple and efficient method for cloning PCR products without the need for restriction enzymes. It relies on the terminal transferase activity of Taq DNA polymerase, which adds a single non-templated deoxyadenosine to the 3' ends of the amplified DNA. This 3'-A overhang can then base-pair with the complementary 3'-T overhangs of a linearized TA cloning vector for easy ligation.

**113.** (C) Deoxyadenosine (A)
During PCR, Taq DNA polymerase often adds an extra nucleotide to the 3' end of the newly synthesized DNA strand in a template-independent manner. This nucleotide is almost always a deoxyadenosine. This property is exploited in TA cloning, where the resulting "A-tailed" PCR products are directly ligated into specialized vectors that have been prepared with complementary single 3'-thymidine overhangs at their ends.

calculation is vital for ensuring that a genomic library is sufficiently large and comprehensive to include all genes.

**133.** (D) Spanning of uncharted DNA regions
Chromosome walking is a method used to find and map genes by sequentially isolating overlapping clones from a genomic library. Starting from a known DNA marker, researchers use the end of one clone as a probe to identify the next adjacent clone in the library. This process is repeated to "walk" along the chromosome, allowing for the characterization of large, uncharted regions of the genome.

**134.** (A) Arrays of repetitive sequences
A major difficulty in chromosome walking occurs when the probe used to find the next clone contains repetitive DNA sequences. Since these sequences are present at many locations throughout the genome, the probe will hybridize to many unrelated clones, leading to false positives and making it impossible to determine the correct path. To overcome this, researchers must ensure their probes consist only of unique DNA.

**135.** (B) Bypassing repetitive DNA regions
Chromosome jumping was developed as a technique to overcome the limitations of chromosome walking, particularly when dealing with large distances or repetitive DNA. Instead of isolating every overlapping clone, chromosome jumping allows researchers to "jump" over several hundred kilobases of DNA. This is achieved by circularizing large fragments and isolating only the junction points, effectively bypassing problematic regions that would otherwise stall a walk.

**136.** (D) Circularization of large fragments
The unique and critical initial step in constructing a chromosome jumping library is the circularization of very large genomic DNA fragments. These fragments, often hundreds of kilobases in size, are ligated under dilute conditions to favor the joining of their own ends. This process brings the distant ends of the fragment together, allowing the intervening DNA to be bypassed in subsequent cloning and mapping steps.

**137.** (C) A suppressor tRNA marker gene
In chromosome jumping, the circularization of large DNA fragments is often facilitated by the inclusion of a suppressor tRNA marker gene, such as supF. When the ends of the large fragment are ligated together, they are joined at this marker. The presence of supF allows for the selective recovery of these junction fragments in specific E. coli host strains, which is essential for identifying the jumping points.

**138.** (C) It isolates only the extreme endpoints of a large region, discarding the middle
Chromosome walking is a systematic process of isolating overlapping clones to build a continuous map of a genomic region. It requires the end of each clone to be used as a probe and necessitates the construction of detailed restriction maps to confirm overlaps. It does not discard the middle of the region; rather, it aims to characterize the entire contiguous sequence, unlike chromosome jumping.

**139.** (B) The extreme distal end of the current clone
To progress in a chromosome walk, a researcher must generate a probe that is specific to the direction of the walk. This is done by isolating a unique DNA sequence from the extreme distal end of the most recently identified clone. This end-probe is then used to screen the genomic library again to find the next overlapping clone that extends further into the uncharted territory.

**140.** (C) I, II, III and IV
The process of chromosome jumping involves several essential steps: first, partial digestion of genomic DNA to produce large fragments; second, circularization of these fragments in the presence of a supF marker; and finally, digestion with a different restriction enzyme to isolate the junction containing the marker. This complex procedure allows researchers to jump over long distances on a chromosome, effectively bypassing repetitive or unclonable regions.

**141.** (B) BACs or YACs
Traditional chromosome walking using plasmid or phage libraries is extremely tedious because of the small insert sizes, requiring many steps to cover a large distance. Modern approaches instead utilize libraries constructed in high-capacity vectors like Bacterial Artificial Chromosomes or Yeast Artificial Chromosomes. These vectors can carry much larger fragments, significantly

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universally used because it complementary base-pairs with the poly-A tail present on nearly all eukaryotic mRNAs. This allows for the synthesis of cDNA from the entire pool of expressed genes in a single reaction.

**124.** (D) Nicking of the mRNA template
In the Gubler-Hoffman method for second-strand cDNA synthesis, RNase H plays a critical role by specifically nicking the mRNA strand within the DNA-RNA hybrid. It does not completely degrade the mRNA but leaves behind small fragments that can serve as primers for DNA polymerase I. This approach is more efficient and less prone to loss of sequence information than older methods using S1 nuclease.

**125.** (B) E. coli DNA Polymerase I
Following the action of RNase H, E. coli DNA polymerase I is used to synthesize the second strand of cDNA. The enzyme uses the small mRNA fragments produced by RNase H as primers and performs nick translation, replacing the RNA with DNA. Finally, DNA ligase seals any remaining nicks to create a complete, double-stranded cDNA molecule that is ready for cloning into a vector.

**126.** (A) Genomic libraries represent the entire genome, while cDNA libraries represent the transcriptome
A genomic library contains the entire DNA sequence of an organism, including coding regions, introns, promoters, and non-coding repetitive DNA. In contrast, a cDNA library is constructed from mRNA and represents only the transcriptome—the genes that were actively being expressed in the specific tissue or at the specific time when the RNA was isolated. Consequently, cDNA clones lack introns and regulatory elements.

**127.** (D) Introns
cDNA is synthesized from mature mRNA, which has already undergone processing in the eukaryotic nucleus. During this processing, introns are spliced out, and a 5' cap and 3' poly-A tail are added. Therefore, a standard cDNA clone will contain the 5' and 3' untranslated regions and the coding sequence but will not contain any intronic sequences that were present in the original genomic DNA.

**128.** (A) Empty phage heads and tail assemblies
In vitro packaging is a technique used to incorporate recombinant Lambda DNA into infectious phage particles. The packaging extracts are derived from E. coli cells and contain all the necessary proteins for phage assembly, including empty heads and tail components. This method is highly efficient for introducing large DNA molecules into bacteria, making it essential for the construction of high-quality genomic and cDNA libraries.

**129.** (C) Partial Sau3AI digestion
To create a representative genomic library, chromosomal DNA must be broken into random, overlapping fragments. This is often achieved through partial digestion with a frequent-cutter restriction enzyme like Sau3AI. By limiting the reaction time or enzyme concentration, researchers can generate a population of fragments that cover the entire genome. These fragments are then size-selected to ensure they match the cloning capacity of the chosen vector.

**130.** (C) I, III and II
The construction of a cDNA library follows a logical sequence. First, mRNA is isolated from total RNA, typically using oligo-dT affinity chromatography. Next, reverse transcription is performed to synthesize the first DNA strand. Finally, the RNA template is removed or nicked by RNase H, and DNA polymerase I is used to synthesize the second DNA strand, resulting in double-stranded cDNA ready for ligation.

**131.** (A) Treatment with EcoRI methylase
When using EcoRI linkers to clone cDNA, it is essential to protect any naturally occurring EcoRI sites within the cDNA itself. This is achieved by treating the double-stranded cDNA with EcoRI methylase before the linkers are ligated. The methylase adds a methyl group to the recognition sites, preventing the EcoRI enzyme from cutting internal sequences when it is used to digest the linkers later.

**132.** (B) Number of clones required for complete genome coverage
The Clarke-Carbon equation is a statistical formula used in genomics to estimate the number of independent clones required to represent the entire genome of an organism with a given probability. It takes into account the genome size and the average size of the cloned fragments. This

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**114.** (D) Transient base-pairing does not occur to stabilize the ends before ligation
Blunt-end ligation is generally less efficient than cohesive-end ligation because there is no transient base-pairing to stabilize the interaction between the DNA ends. In the absence of complementary overhangs, the ligase must capture two DNA molecules that are only held together by random collisions. Consequently, this reaction typically requires higher concentrations of both the DNA fragments and the T4 DNA ligase enzyme.

**115.** (A) Adapters have pre-formed sticky ends
DNA linkers and adapters are synthetic oligonucleotides used to facilitate cloning. Linkers are short, double-stranded blunt-ended molecules that contain a restriction site. Once ligated to blunt-ended DNA, they must be digested to create sticky ends. In contrast, adapters are pre-formed with one blunt end and one sticky end, allowing them to be ligated directly to DNA without the need for subsequent digestion.

**116.** (B) Restriction and ligation steps
Gateway technology is a powerful cloning method that bypasses the traditional requirements for restriction enzymes and DNA ligase. Instead, it utilizes site-specific recombination based on the integration system of requesting bacteriophage Lambda. This allows for the rapid and efficient transfer of DNA fragments between different vectors while maintaining the correct reading frame and orientation, greatly simplifying the construction of complex expression clones.

**117.** (D) attB and attP
In the Gateway cloning system, the initial step involves the creation of an Entry Clone through the BP reaction. This reaction is catalyzed by the BP clonase enzyme mix, which facilitates site-specific recombination between attB sites on a PCR product and attP sites on a Donor Vector. This results in the target gene being flanked by attL sites, ready for further transfer into various destination vectors.

**118.** (D) attL and attR sites
The second step of the Gateway cloning process is the LR reaction, which transfers the gene of interest from an Entry Clone to a Destination Vector. This reaction is mediated by the LR clonase enzyme mix, which recognizes and acts upon the attL sites in the Entry Clone and the attR sites in the Destination Vector. This precise recombination creates the final Expression Clone.

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**119.** (C) ccdB
The Gateway cloning system uses the ccdB gene as a negative selection marker to ensure high efficiency. The Destination Vector contains this lethal gene between its attR sites. During the LR reaction, the ccdB gene is replaced by the gene of interest. Any bacteria that take up an un-recombined Destination Vector will be killed by the CcdB toxin, leaving only the desired recombinants.

**120.** (A) P-2, Q-3, R-4, S-1
Various cloning techniques offer unique advantages. TA cloning utilizes the 3'-A overhangs produced by Taq polymerase. Directional cloning employs two different restriction enzymes for precise orientation. Blunt-end ligation is versatile but less efficient, requiring more ligase. Finally, Gateway cloning represents a modern approach that uses site-specific recombination to move DNA between vectors without the need for traditional restriction and ligation steps.

**121.** (C) Alkaline phosphatase
When performing non-directional cloning with a single restriction enzyme, the linearized vector can easily recircularize during the ligation reaction. To prevent this, the vector is treated with alkaline phosphatase to remove the 5'-phosphate groups. Since DNA ligase requires a 5'-phosphate to form a phosphodiester bond, the dephosphorylated vector cannot self-ligate, forcing it to ligate with the phosphorylated insert DNA instead.

**122.** (A) Oligo-dT cellulose
To construct a cDNA library, it is necessary to isolate mRNA from the much more abundant ribosomal and transfer RNA. This is achieved by exploiting the poly-A tail found at the 3' end of most eukaryotic mRNAs. Total RNA is passed through a column containing oligo-dT cellulose. The poly-A tails base-pair with the oligo-dT, allowing the mRNA to be retained while other RNA types are washed away.

**123.** (C) Oligo-dT
First-strand cDNA synthesis is catalyzed by reverse transcriptase using mRNA as a template. To initiate this process, a primer must be annealed to the mRNA. An oligo-dT primer is

reducing the number of steps needed to walk across a large genomic region.

**142.** (B) It requires only one round of library screening to walk 1000 kb in a cosmid library
Chromosome walking is a multi-step process that typically requires many rounds of library screening to cover a significant distance. Each step only moves forward by the size of the overlap between clones. In a cosmid library, where inserts are about forty-five kilobases, it would require dozens of rounds to walk one thousand kilobases. The process also demands careful restriction mapping and the avoidance of repetitive DNA.

**143.** (C) Amber nonsense mutation suppression
The supF gene encodes a suppressor tRNA that can overcome amber nonsense mutations in the host cell's genes. In chromosome jumping, this marker is used to selectively recover only those clones that contain the junction of the circularized large DNA fragment. When the library is plated on a host with amber mutations in essential genes, only the recombinants carrying the supF gene will survive.

**144.** (A) Sugars and phenolic compounds
The first stage of Agrobacterium-mediated transformation is the movement of the bacteria toward wounded plant cells. This chemotactic response is triggered by the release of various compounds from the damaged tissue, including sugars and phenolic substances. These molecules act as signals that attract the Agrobacterium and prepare it for the subsequent steps of attaching to the plant cell and initiating T-DNA transfer.

**145.** (D) VirA and VirG
The activation of the virulence system in Agrobacterium is controlled by the VirA and VirG proteins, which form a two-component regulatory system. VirA is a membrane-bound sensor kinase that detects phenolic compounds like acetosyringone. Upon detection, VirA autophosphorylates and then transfers the phosphate to VirG, a transcriptional activator. Phosphorylated VirG then induces the expression of the other vir operons needed for transformation.

**146.** (B) VirB operon proteins and VirD4
The transfer of the T-DNA complex from Agrobacterium into the host plant cell is mediated by a specialized Type IV secretion system. This complex structure is primarily composed of proteins encoded by the virB operon, along with the VirD4 protein. Together, they form a channel that spans both bacterial membranes and the plant cell wall, allowing for the direct export of the T-strand.

**147.** (A) Protection of the single T-strand
VirE2 is a critical single-stranded DNA binding protein produced by Agrobacterium. Its primary role is to coat and protect the single-stranded T-strand from degradation by nucleases as it moves through the plant cell cytoplasm. Additionally, VirE2 helps to keep the T-strand in an unfolded, linear conformation, which is necessary for its efficient transport into the plant nucleus through the nuclear pore complexes.

**148.** (D) VIP1
In the host plant cell, the Agrobacterium T-complex interacts with several cellular proteins to facilitate its journey to the nucleus. One key player is VIP1, a plant protein that specifically binds to VirE2. VIP1 acts as a bridge, linking the T-complex to the plant's own nuclear import machinery. This interaction is essential for the successful delivery of the T-DNA into the plant's genomic environment.

**149.** (C) vir operon
For Agrobacterium to mobilize T-DNA, the T-DNA region must be flanked by specific border sequences. Of these, the Right border is absolutely required in cis, meaning it must be physically located on the same DNA molecule as the genes to be transferred. While the vir genes are essential, they can function in trans from a separate plasmid, but the Right border defines the T-DNA.

**150.** (C) I, II, III, IV and V
Agrobacterium-mediated transformation follows a precise chronological sequence. It begins with the sensing of acetosyringone by VirA, followed by the activation of VirG through phosphorylation. Once active, VirG induces T-DNA processing by the VirD1/VirD2 complex. The resulting T-strand is then exported via the VirB/D4 secretion system and finally imported into the plant nucleus, guided by specific nuclear localization signals on the attached proteins.

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with biotin, digoxigenin, or radioactive isotopes. The goal is to create a labeled molecule that can base-pair with the target DNA. The statement that the "target DNA probe incorporation" is incorrect refers to the fact that the probe is the labeled molecule, not the target itself.

**188.** (A) RNA
Northern hybridization is a laboratory technique used to study gene expression by detecting specific RNA molecules in a sample. Total RNA or mRNA is separated by size using electrophoresis, transferred to a membrane, and then hybridized with a labeled DNA or RNA probe. This allows researchers to determine the size and relative abundance of a particular transcript in different tissues or under various conditions.

**189.** (A) Exponential phase real-time monitoring
Quantitative real-time RT-PCR is a highly sensitive method for measuring gene expression. It involves the reverse transcription of RNA into cDNA, followed by PCR amplification. The amount of product is monitored in real-time during the exponential phase of the reaction using fluorescent dyes or probes. This allows for the precise quantification of the initial amount of target mRNA present in the original biological sample.

**190.** (C) II → III → IV → I
A Western blot assay follows a specific sequence of steps: first, proteins are separated by size using SDS-PAGE; second, the proteins are transferred to a PVDF or nitrocellulose membrane; third, the membrane is incubated with a primary antibody that binds to the target protein; and finally, a secondary antibody is added for detection. This process allows for the identification and quantification of specific proteins.

**191.** (C) The target transgenic protein
In a Western blot, the primary antibody is the most critical component for specificity. It is designed to recognize and bind only to a specific epitope on the target transgenic protein. After the proteins have been transferred to the membrane and the non-specific binding sites have been blocked, the primary antibody is applied. Its binding marks the exact location of the target protein on the blot.

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**192.** (B) High-throughput quantification
Enzyme-Linked Immunosorbent Assay is a powerful tool for analyzing transgenic plants because it allows for the high-throughput, quantitative detection of recombinant proteins. ELISA can be performed in ninety-six-well plates, making it possible to screen hundreds of samples simultaneously. It is particularly useful for measuring the levels of transgene expression across different lines and for monitoring the concentration of GM proteins in commercial crops.

**193.** (B) Precise spatial expression mapping
Immunohistochemical localization is a technique used to determine the precise spatial distribution of a protein within a tissue or cell. It involves using labeled antibodies that bind to the target protein in fixed tissue sections. By visualizing the label under a microscope, researchers can see exactly which cells and which sub-cellular compartments are expressing the transgene, providing valuable information about its function and regulation.

**194.** (D) Northern Blot
To confirm the size and presence of a recombinant protein, techniques such as SDS-PAGE, Western blotting, and mass spectrometry are commonly used. These methods provide information about the molecular weight and amino acid composition of the protein. Northern blotting, however, is used to detect RNA molecules and cannot provide any direct information about the size or properties of the synthesized protein product.

**195.** (C) All double-stranded DNA generated
SYBR Green I is a fluorescent dye widely used in real-time PCR for the non-specific detection of amplification products. The dye exhibits a massive increase in fluorescence only when it is bound to the minor groove of double-stranded DNA. As the PCR reaction progresses and the amount of double-stranded product increases, the fluorescent signal rises proportionally, allowing for the real-time monitoring of the reaction.

**196.** (A) Stable internal reference (housekeeping) genes
Accurate quantification in qRT-PCR requires normalization to control for variations in RNA quality and quantity between samples. This is achieved by measuring the expression of stable internal reference genes, often called

**160.** (A) Both A and R are true and R is the correct explanation of A.
Particle bombardment is the preferred method for chloroplast transformation because it can physically deliver DNA directly into the plastids. Unlike the Agrobacterium system, which evolved to specifically target the plant cell nucleus, biolistics is non-biological and can project DNA into any cellular compartment. This capability is essential for engineering the chloroplast genome, which requires high-velocity penetration of the double-membrane organelle for successful integration.

**161.** (A) Fragile protoplast isolation
A major limitation of PEG-mediated transformation is its absolute requirement for protoplasts, which are plant cells that have had their cell walls enzymatically removed. Protoplasts are extremely fragile and difficult to handle. Furthermore, regenerating entire fertile plants from single protoplasts is a complex and often inefficient process that is not yet possible for many important crop species, limiting the general utility of this method.

**162.** (A) The burst rating of the rupture disk
In a helium-driven gene gun, the exact pressure of the gas pulse is controlled by a specialized component known as a rupture disk. These disks are designed to burst at a specific, predetermined pressure. By selecting disks with different burst ratings, researchers can precisely calibrate the acceleration of the microparticles to match the requirements of the specific target tissue, ensuring optimal penetration and transformation efficiency.

**163.** (C) Obstruction of the macrocarrier
The stopping screen in a biolistic device is a critical mechanical component positioned between the macrocarrier and the target tissue. Its function is to abruptly halt the forward motion of the macrocarrier after it has been accelerated by the gas pulse. While the macrocarrier is caught, the DNA-coated microparticles pass through the holes in the screen and continue toward the cells, preventing the macrocarrier from damaging the sample.

**164.** (B) Cell wall
PEG-mediated transformation cannot be performed on intact plant cells because the rigid cell wall acts as a barrier to DNA entry. Therefore, the cell wall must be enzymatically digested using cellulases and pectinases to produce spherical, membrane-bound protoplasts. Once the wall is removed, the plasma membrane is exposed, allowing PEG to induce the necessary permeability for the DNA to enter the cytoplasm.

**165.** (C) Duration of the electrical pulse
To achieve successful transformation using biolistics, several parameters must be carefully optimized, including the target distance, helium pressure, and vacuum level in the chamber. These factors affect the velocity and spread of the microparticles. However, the duration of an electrical pulse is not a parameter for standard helium-driven gene guns, as it is a characteristic of electroporation or discharge-driven systems instead.

**166.** (B) Unfertilized female germline
The floral dip method is a highly successful in planta transformation technique, particularly for Arabidopsis. It involves dipping developing flowers into a suspension of Agrobacterium. The bacteria enter the female reproductive organs and transform the unfertilized ovules. Because the germline itself is transformed, the resulting seeds are transgenic, allowing for the direct recovery of modified offspring without the need for complex tissue culture.

**167.** (B) Removal of intercellular air
Vacuum infiltration is used in plant transformation to improve the contact between Agrobacterium and the target tissues. By applying a vacuum, air is drawn out of the intercellular spaces within the plant organs. When the vacuum is released, the surrounding bacterial suspension is pulled into these spaces, ensuring that the Agrobacterium reaches a larger number of cells, thereby increasing the overall efficiency of the transformation.

**168.** (D) Reduction of surface tension
In the floral dip protocol, surfactants like Silwet L-77 are added to the Agrobacterium inoculation medium to reduce the surface tension of the liquid. This allows the bacterial suspension to spread more easily and penetrate deep into the narrow spaces of the floral buds, specifically reaching the developing ovules. This improvement in coverage is essential for achieving high rates of transformation in the germline.

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**151.** (C) Non-homologous end joining (NHEJ)
Once the T-DNA reaches the plant nucleus, it must integrate into the host genome. This process is not well understood but is believed to rely predominantly on the plant's own non-homologous end joining DNA repair pathway. Unlike homologous recombination, NHEJ does not require sequence similarity between the T-DNA and the host genome, allowing the T-DNA to integrate at more or less random locations.

**152.** (A) virA/virG or virG/virE
Supervirulent strains of Agrobacterium, which are more efficient at transforming recalcitrant plant species, often contain modified Ti plasmids or extra plasmids. These additional genetic elements frequently provide extra copies of the virA and virG genes, or sometimes virG and virE. Increasing the dosage of these key regulatory and protective proteins enhances the bacterium's overall ability to process and deliver T-DNA into the host.

**153.** (A) Enhancement of vir gene induction
While phenolic compounds like acetosyringone are the primary inducers of the Agrobacterium vir operon, certain sugars such as glucose and galactose also play a significant role. These sugars enhance the sensitivity of the VirA sensor, allowing for the induction of virulence genes even at very low concentrations of phenolics. This cooperative effect significantly improves the efficiency of infection and transformation in many plant species.

**154.** (C) The synthesis of replacement DNA on the Ti plasmid requires RNA polymerase
During T-DNA processing, the VirD1/VirD2 complex produces a single-stranded T-strand. VirD2 remains attached to the 5' end as it is displaced by new DNA synthesis on the Ti plasmid. This synthesis is performed by DNA polymerase, not RNA polymerase. The T-strand is generated in a polar fashion, starting from the right border and proceeding toward the left, ensuring the entire region is accurately copied.

**155.** (B) High-pressure helium gas
Particle bombardment, also known as biolistics, is a physical method for delivering DNA into cells. In modern gene gun systems, the force required to accelerate the microparticles is provided by a burst of high-pressure helium gas. This gas pulse is released suddenly, propelling a macrocarrier or the particles themselves toward the target tissue with enough velocity to penetrate the cell walls and membranes.

**156.** (A) Gold or tungsten
The microcarriers used in biolistics must be dense, chemically inert, and small enough to enter cells without causing excessive damage. Gold and tungsten are the most commonly used materials for this purpose. DNA is precipitated onto the surface of these metal spheres, which are then accelerated into the target tissue. Gold is often preferred over tungsten because it is less toxic to plant cells.

**157.** (B) Acceleration of the microparticles
In many biolistic systems, the microparticles are initially loaded onto a larger plastic disk called a macrocarrier. When the high-pressure gas pulse is released, it accelerates the macrocarrier down the barrel of the device. The macrocarrier is then abruptly stopped by a screen, while the much smaller microparticles continue their flight, carrying the DNA into the target plant tissues at high speed.

**158.** (C) Reversible membrane permeability
Polyethylene glycol is used in direct gene transfer to facilitate the uptake of DNA by plant protoplasts. PEG acts by altering the physical properties of the plasma membrane, inducing reversible permeability. Under specific concentrations and conditions, PEG causes the membrane to become leaky or facilitates the fusion of DNA-calcium phosphate precipitates with the membrane, allowing the DNA to enter the cytoplasm through a non-mechanical process.

**159.** (B) Mechanical piercing via agitation
Silicon carbide whisker-mediated transformation is a simple technique where DNA and plant cells are agitated together with small, needle-like fibers. These whiskers, which are very hard and sharp, physically pierce the cell walls and membranes during the mixing process. This creates transient openings through which the DNA can enter the cells. This method is particularly useful for transforming certain monocot species and suspension cultures.

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**178.** (B) Isolation of flanking sequences
Thermal Asymmetric Interlaced PCR is a specialized technique used to recover the genomic DNA sequences that flank a known T-DNA or transposon insertion. By amplifying the region between the known insert and the unknown neighboring DNA, researchers can determine the exact location of the transgene in the host genome. This is essential for studying integration site effects and for developing event-specific molecular markers.

**179.** (A) Specific nested and degenerate primers
TAIL-PCR utilizes a combination of three nested, sequence-specific primers and a shorter, degenerate primer with a lower melting temperature. The reaction involves several cycles of high and low stringency to selectively amplify the target sequence while suppressing non-specific products. This "interlaced" thermal profile ensures that the specific product is enriched over background noise, allowing for the efficient isolation of flanking genomic DNA.

**180.** (A) II → IV → I → III
The Southern blotting procedure follows a set sequence: first, genomic DNA is digested with restriction enzymes; second, the fragments are separated by agarose gel electrophoresis; third, the DNA is transferred from the gel to a membrane; and finally, the membrane is hybridized with a labeled probe. This allows for the detection of specific DNA sequences within a complex mixture, such as a transgene.

**181.** (A) Improved large fragment transfer
Treating an agarose gel with dilute hydrochloric acid before Southern transfer is known as depurination. This step introduces small nicks in the DNA backbone, which is particularly important for the efficient transfer of very large DNA fragments. Smaller fragments move more easily from the gel to the membrane, ensuring that the entire range of DNA sizes is represented on the blot for subsequent hybridization.

**182.** (A) Substrate-to-light conversion
Chemiluminescent detection is a highly sensitive, non-radioactive method for visualizing hybridized probes in Southern or Western blotting. It involves an enzyme-linked probe that catalyzes the conversion of a specific substrate into a light-emitting product. The light is then detected using X-ray film or a digital imaging system. This technique offers high resolution and sensitivity while avoiding the safety and disposal issues associated with isotopes.

**183.** (D) Gas-phase bombardment
Several methods are used to transfer DNA from a gel to a membrane, including capillary transfer, where buffer moves by wicking action, electrophoretic transfer, and vacuum transfer. Each of these techniques aims to move the DNA molecules while maintaining their relative positions. Gas-phase bombardment, however, is a method for delivering DNA into living cells and is not used in the blotting process.

**184.** (A) Persisting Agrobacterium residue
A common source of false positives in the PCR-based validation of transgenic plants is the persistence of Agrobacterium in the treated tissues. Even if the bacteria are no longer active, their DNA can remain and be amplified by the transgene-specific primers, leading to the mistaken conclusion that the plant is transformed. To avoid this, researchers must use antibiotics to eliminate bacteria and perform thorough checks.

**185.** (A) Once within the T-DNA region
To determine the transgene copy number using Southern blot, a restriction enzyme that cuts only once within the T-DNA region is typically used. This ensures that each integration site produces a single fragment of a unique size, determined by the distance to the next restriction site in the host genome. By counting the number of bands on the blot, researchers can accurately identify single-copy events.

**186.** (B) Event-specific marker development
The flanking sequence data obtained through techniques like TAIL-PCR is essential for the development of event-specific markers. These markers are unique to a particular transgenic line because they span the junction between the T-DNA and the host genome. They are used for the precise identification and monitoring of specific GM events in breeding programs, regulatory inspections, and for tracking the transgene in the environment.

**187.** (D) Target DNA probe incorporation
Probes for hybridization are labeled using various methods, such as random primer extension or nick translation, incorporating nucleotides tagged

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**169.** (A) Flanking homologous sequences
Vectors for chloroplast transformation are fundamentally different from nuclear vectors. They must include flanking sequences that are homologous to the targeted region of the plastid genome. This design enables the transgene to be integrated at a precise location through the process of homologous recombination, which is highly efficient in chloroplasts. This site-specific integration avoids the random position effects often seen in nuclear transformation.

**170.** (C) P-2, Q-4, R-3, S-1
Different plant transformation methods have unique requirements. Floral dip is an in planta method that avoids tissue culture. Biolistics uses high-pressure helium to deliver DNA and is often used for plastids. PEG-mediated transformation requires the isolation of protoplasts. Finally, plastid transformation is characterized by its reliance on homologous recombination for site-specific integration, offering a high degree of precision in genetic engineering.

**171.** (A) Maternal inheritance pattern
Transgenes integrated into the chloroplast genome typically follow a pattern of maternal inheritance in most crop species. This means they are passed on through the seeds but are not present in the pollen grains. This trait is highly advantageous for biosafety because it virtually eliminates the risk of transgene escape through pollen-mediated outcrossing to wild relatives or neighboring non-GM crops.

**172.** (A) Extremely low expression levels
Plastid transformation offers several significant advantages, including the ability to express multiple genes as operons and the avoidance of gene silencing. Contrary to the idea of low expression, chloroplasts can produce extremely high levels of recombinant proteins, sometimes exceeding forty percent of total leaf protein. Achieving homoplasmy, where all plastid genomes carry the transgene, is essential for stable inheritance and high-level expression.

**173.** (A) Transgenes in all plastid genomes
Homoplasmy is a critical state in chloroplast transformation where every single copy of the plastid genome within a cell contains the transgene. Since a single plant cell can contain thousands of copies of the chloroplast genome, several rounds of selection and regeneration are necessary to replace all wild-type genomes with the modified ones. Homoplasmy ensures the stable inheritance and uniform expression of the transgene.

**174.** (A) Avoidance of somaclonal variation
The primary benefit of tissue-culture independent methods, like the floral dip, is the avoidance of somaclonal variation. Traditional transformation techniques involve long periods of in vitro regeneration, which can induce unintended genetic and epigenetic changes in the plant. By bypassing these steps and transforming the germline directly, researchers can produce transgenic plants that are more genetically similar to the original parent line.

**175.** (B) Strong endogenous plastid promoters
Promoters used in chloroplast expression cassettes, such as psbA or the rrn operon promoter, are derived from the chloroplast's own genome. These strong endogenous promoters are recognized by the plastid's unique transcription machinery, which is more similar to that of bacteria than to the eukaryotic nucleus. Using these promoters ensures the efficient and high-level production of recombinant mRNA within the organelle.

**176.** (D) The resulting seeds are all transgenic
While the floral dip method is highly efficient for Arabidopsis thaliana, it does not result in a population where all the seeds are transgenic. In reality, only a small percentage of the seeds, typically around one to five percent, actually carry the transgene. Therefore, a critical subsequent step is to plate the harvested seeds on selective media to identify and recover the few true transformants.

NODIA

**177.** (A) The precise integration pattern and copy number of the transgene
Southern hybridization is a fundamental technique for characterizing transgenic plants. By digesting genomic DNA and probing for the transgene, researchers can determine the exact number of integration events (copy number) and identify the specific pattern of integration. This information is crucial for confirming that the transgene is present as a stable, intact unit and for selecting lines with simple, single-copy insertions.

housekeeping genes, such as actin or GAPDH. By comparing the target gene's signal to that of the reference gene, researchers can correct for experimental errors and determine the true relative expression level.

**197.** (B) Taq polymerase
A TaqMan probe is a sequence-specific oligonucleotide labeled with a fluorophore and a quencher. During the annealing phase of PCR, the probe binds to the target DNA. As Taq polymerase extends the new strand, its inherent 5' to 3' exonuclease activity degrades the probe, separating the fluorophore from the quencher. This results in an increase in fluorescence that is proportional to the amount of DNA.

**198.** (D) Native 3D conformation retention
Western blotting involves the separation of proteins using SDS-PAGE, which typically denatures the proteins to ensure they separate based on their molecular weight rather than their shape. Therefore, the target protein does not maintain its native three-dimensional conformation during the process. The detection relies on the binding of antibodies to specific epitopes, which are often exposed only when the protein is in a denatured state.

**199.** (C) Chemical and nutritional similarity
The concept of substantial equivalence is a cornerstone of GMO risk assessment. It posits that if a new genetically modified food is found to be chemically and nutritionally similar to its traditional counterpart, it can be considered as safe as the conventional version. This assessment involves comparing key components such as proteins, fats, vitamins, and minerals to identify any unintended changes resulting from the genetic modification.

**200.** (A) Chloroplast transgene engineering
Biological containment strategies aim to prevent the unintended spread of transgenes into the environment. One effective method is to engineer the transgene into the chloroplast genome rather than the nucleus. Because chloroplasts are maternally inherited in many crops, the transgene is not present in the pollen and cannot be transferred to wild relatives through cross-pollination. This significantly reduces the risk of gene flow and environmental impact.

**201.** (A) Stability in simulated gastric fluid
To assess the potential allergenicity of a novel transgenic protein, researchers often test its stability in simulated gastric fluid. Most known food allergens are resistant to digestion by pepsin under acidic conditions. If a transgenic protein is rapidly broken down in the stomach, it is considered to have a lower risk of causing an allergic reaction. This biochemical test is a standard part of the safety evaluation.

**202.** (A) Bt impact on non-target insects
Non-target effects refer to the unintended impact of a transgenic trait, such as the insecticidal Bt protein, on organisms that are not the intended pests. Risk assessment involves evaluating whether the Bt protein is toxic to beneficial insects, like pollinators or natural predators. Understanding these effects is essential for ensuring that the use of GM crops does not disrupt the broader ecosystem or lead to a decline in biodiversity.

**203.** (A) Biosafety Level 1 (BSL-1)
Biosafety Level 1 is the lowest level of containment and is appropriate for work involving well-characterized biological agents that are not known to consistently cause disease in healthy adult humans. These agents pose minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories do not require special isolation and typically involve standard microbiological practices, such as hand washing and the use of basic protective equipment.

NODIA

**204.** (B) Open-window laboratory benches
Biosafety Level 4 is the highest level of containment, reserved for dangerous and exotic agents that pose a high risk of life-threatening disease. Requirements include positive pressure suits, HEPA-filtered air supplies, and strict isolation from general areas. Open-window laboratory benches are strictly prohibited and completely incompatible with BSL-4 standards, as they would allow the uncontrolled release of highly infectious and potentially lethal biological agents.

**205.** (B) BSL-3
Biosafety Level 3 is required for work with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation. This level involves strict laboratory design and practices, such as the use of biological

**223.** (D) 20 years
In India, the standard term for a patent is twenty years from the date of filing the application. This duration is consistent with the international standards set by the TRIPS agreement. During this period, the patent holder has the exclusive right to prevent others from making, using, selling, or importing the patented invention. After the term expires, the invention enters the public domain and can be used by anyone.

**224.** (A) Genetically modified microorganisms with human intervention and utility are generally patentable
Genetically modified microorganisms are generally patentable in India, provided they meet the criteria of novelty, inventive step, and industrial utility. While naturally occurring microbes cannot be patented, those that have been significantly altered by human intervention to provide a new function are eligible for protection. This reflects the global shift toward recognizing the intellectual property value of innovative biotechnological research involving microbial life forms for various industrial applications.

**225.** (B) WTO
The Agreement on Trade-Related Aspects of Intellectual Property Rights is an international treaty that sets minimum standards for the regulation of intellectual property. It is administered by the World Trade Organization. TRIPS requires member nations to provide patent protection for inventions in all fields of technology, including biotechnology, while allowing for certain exceptions for plants, animals, and essential biological processes, balancing private rights with public interests.

**226.** (B) A traditional method of agriculture or horticulture
While novel vectors and genetically engineered bacteria can be patented in India, traditional methods of agriculture or horticulture are explicitly excluded. This is part of the Indian Patent Act's effort to protect traditional knowledge and the rights of farmers. Such methods are considered part of the common heritage and cannot be claimed as private intellectual property, ensuring that traditional farming practices remain free for everyone to use and develop.

**227.** (A) Compulsory licensing
Compulsory licensing is a mechanism within patent law that allows the government to authorize a third party to produce a patented product without the owner's consent. In India, this can be triggered by public health emergencies or if the patented invention is not available to the public at an affordable price. This provision ensures that the monopoly granted by a patent does not override the fundamental right to access life-saving medicines or technologies.

**228.** (C) Genomic DNA is ineligible; cDNA is eligible
The legal landscape for gene patenting has evolved significantly. In the United States, following the Myriad Genetics ruling, genomic DNA in its natural state is considered a product of nature and is not patentable. However, synthetic DNA, such as cDNA created in the laboratory, is eligible for patent protection because it does not exist naturally in that form. This distinction has had a profound impact on the biotechnology and diagnostic industries.

**229.** (A) Deposit in international authorities
The Budapest Treaty is an international agreement that simplifies the patent process for inventions involving microorganisms. It allows an inventor to deposit a sample of the microbe in a single recognized international depository authority, which then serves all member countries. This replaces the need to deposit a sample in every country where a patent is sought, making the protection of microbial inventions more efficient and cost-effective for researchers.

**230.** (A) I → IV → III → II
The process of obtaining a patent follows a chronological sequence: first, the application is filed with the patent office; second, the application is published in the official journal; third, a formal examination is conducted to verify if the invention meets all the legal criteria; and finally, the patent is either granted or refused. This structured procedure ensures that only truly innovative and useful inventions receive legal protection.

**231.** (A) Establish breeder intellectual property rights
The International Union for the Protection of New Varieties of Plants (UPOV) is an intergovernmental organization that aims to provide and promote an effective system of plant variety protection. Its goal is to encourage the

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the unintended spread of the transgene. One mandatory measure is the establishment of isolation distances from other compatible plant species. These trials must be approved by the RCGM and are closely monitored to ensure that the GM material is handled safely and that no transgenic seeds or pollen escape into the surrounding environment.

**215.** (A) District Level Committee (DLC)
At the district level, the District Level Committee is responsible for monitoring the safety regulations of genetically modified organisms and ensuring compliance with the national guidelines. The DLC is headed by the District Magistrate and includes representatives from various local departments. Its role is to oversee the implementation of biosafety measures in the field and to investigate any reports of unauthorized or unsafe activities involving GMOs.

**216.** (C) Low-risk basic laboratory projects
The GEAC is responsible for high-level regulatory decisions, including the large-scale release of GMOs, the import of GM products, and the final commercialization of transgenic crops. However, basic laboratory projects that are well-characterized and carry low biosafety risks typically fall under the purview of the Institutional Biosafety Committee or the RCGM. These lower-level bodies handle the routine monitoring and approval of preliminary research before it reaches the GEAC.

**217.** (A) 1986
The regulatory framework for biosafety in India was established under the Environment (Protection) Act of 1986. This landmark legislation provided the legal basis for the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Micro-organisms, Genetically Engineered Organisms or Cells," which were promulgated in 1989. These rules define the roles of the various committees like IBSC, RCGM, and GEAC in managing the risks of biotechnology.

**218.** (A) Event-by-event basis
In India's regulatory system, the approval for a transgenic crop is granted on an "event-by-event" basis. This means that each specific case of a transgene being inserted into a particular location in the host genome is treated as a unique entity. Even if the same gene is used in a different variety or a different crop, it must undergo a separate and comprehensive safety assessment before approval.

**219.** (A) State-level biosafety monitoring
The State Biotechnology Coordination Committee is a state-level regulatory body in India responsible for coordinating and monitoring activities related to the use of GMOs within the state. The SBCC has the power to inspect field trials and ensure that the safety guidelines are being followed. It serves as a vital link between the national regulatory authorities and the local administration, helping to enforce biosafety compliance.

NODIA

**220.** (A) P-2, Q-4, R-1, S-3
India's biosafety framework involves multiple layers of oversight. The Institutional Biosafety Committee (IBSC) monitors research at the institute level. The Review Committee on Genetic Manipulation (RCGM) approves national-level confined field trials. The Genetic Engineering Appraisal Committee (GEAC) grants commercial approvals for large-scale release. Finally, the District Level Committee (DLC) ensures compliance at the local level, creating a comprehensive system for managing the risks of biotechnology.

**221.** (B) Industrial applicability (utility)
To be eligible for a patent in India, an invention must be novel, involve an inventive step (non-obviousness), and possess industrial applicability or utility. Novelty means the invention must not have been previously disclosed. The inventive step ensures it is not an obvious development to someone skilled in the art. Finally, utility requires that the invention can be made or used in an industry, providing a practical benefit.

**222.** (A) Whole plants, animals, or seeds
Section 3(j) of the Indian Patent Act is a critical provision that defines what is not patentable. It specifically excludes plants and animals in whole or in any part thereof, including seeds, varieties, and species, and essentially biological processes for their production. This means that while a modified gene or a specific process might be patentable, the whole transgenic organism itself cannot be owned under the patent system in India.

Reason (R): Inosine is recognized as guanosine by the translational machinery, thus changing the codon meaning.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2012

**19.** Identify the correct sequence of events in the siRNA-mediated RNA interference pathway.
(A) Dicer cleavage → dsRNA entry → RISC assembly → Target mRNA cleavage
(B) dsRNA entry → Dicer cleavage → RISC assembly → Target mRNA cleavage
(C) RISC assembly → dsRNA entry → Dicer cleavage → Target mRNA cleavage
(D) dsRNA entry → RISC assembly → Dicer cleavage → Target mRNA cleavage

2012

**20.** Given below are certain statements about plant bioprospecting via metagenomics.
I. It bypasses the need for culturing extreme environment microbes.
II. It relies solely on phenotypic screening of whole plants.
III. It facilitates the discovery of novel bioactive molecules.
IV. It only utilizes 16S rRNA gene amplification without whole-genome sequencing.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) II, III and IV
(C) I, III and IV
(D) I and III

2015

**21.** What defines the primary strategy of genetic biofortification in Golden Rice?
(A) Downregulation of the ferritin degradation pathway
(B) Insertion of the phytoene synthase and carotene desaturase genes
(C) Overexpression of nicotianamine synthase to bind zinc
(D) Engineering the chloroplast genome to produce ascorbic acid

2005

**22.** Which molecular tool is necessary for confirming successful gene pyramiding of multiple resistance alleles in a crop?
(A) Northern blotting of secreted root exudates.
(B) Agrobacterium tumefaciens mediated co-cultivation.
(C) Marker-assisted selection using locus-specific markers.
(D) Protoplast fusion without any prior selection steps.

2004

**23.** Identify the correct pair regarding gene fusion systems and their primary applications.
(A) GUS - Intracellular protein degradation targeting
(B) His-tag - Enhancing the transcriptional rate of the promoter
(C) Luciferase - Facilitating site-directed mutagenesis
(D) GFP - Real-time in vivo sub-cellular localization

2022

**24.** Which structure defines a hammerhead ribozyme?
(A) A small catalytic RNA motif comprising three base-paired helices converging on a core
(B) A lariat-forming intron complex that facilitates the first transesterification step
(C) A protein-RNA complex utilizing a zinc ion for structural stability and catalysis
(D) A highly structured poly-A tail sequence required for enzymatic transcript cleavage

2006

**25.** Match List I with List II.
List I: (Translational Inhibitors)
P. Tetracycline
Q. Chloramphenicol
R. Cycloheximide
S. Diphtheria toxin
List II: (Target Domains)
1. Eukaryotic eEF2
2. Eukaryotic 60S peptidyl transferase
3. Prokaryotic 50S peptidyl transferase
4. Prokaryotic 30S A-site
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-1, Q-2, R-3, S-4
(D) P-2, Q-1, R-4, S-3

2007

**26.** Identify the INCORRECT pair regarding translation mechanisms.
(A) $eEF_1A$ - Delivers aminoacyl-tRNA to the A-site in eukaryotes

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development of new varieties of plants for the benefit of society by establishing intellectual property rights for breeders. This system incentivizes investment in plant breeding, leading to improved crops with better yield, quality, and resistance to pests.

**232.** (A) Distinctness, Uniformity, Stability
To be eligible for protection under plant variety rights, a new variety must satisfy the DUS criteria: Distinctness, Uniformity, and Stability. Distinctness means the variety must be clearly distinguishable from any other commonly known variety. Uniformity requires that all individual plants in the variety share the same characteristics. Stability ensures that the variety's essential traits remain unchanged over successive generations of propagation, providing a reliable basis for intellectual property protection.

**233.** (A) 2001
The Protection of Plant Varieties and Farmers' Rights Act was enacted by the Indian Parliament in 2001. This legislation was developed in response to the TRIPS agreement but is unique in its focus on balancing the rights of plant breeders with the rights of farmers. It provides a legal framework for protecting new plant varieties while simultaneously recognizing the historical role of farmers as conservers and breeders of genetic resources.

**234.** (A) Freedom to save and sell farm seed
A key feature of the Indian PPV&FR Act is the explicit recognition of farmers' rights. This includes the freedom to save, use, sow, resow, exchange, share, or sell their farm-saved seed, even of a variety protected under the Act. However, farmers are not allowed to sell "branded" seed that uses the breeder's name. This provision ensures that traditional farming practices can continue while still providing protection for modern plant breeding.

**235.** (A) Use a protected variety as a source of variation for new breeds
The PPV&FR Act includes "Researchers' Rights," which allow scientists to use a protected variety for the purpose of conducting research or as an initial source of variation for creating a new variety. This "breeder's exemption" is vital for ensuring that intellectual property rights do not stifle further innovation. It allows the continuous improvement of plant breeds by providing researchers with access to the best available genetic material for their work.

**236.** (D) Commercial profitability
To register a plant variety under the PPV&FR Act, it must meet the legal requirements of novelty, distinctness, uniformity, and stability. These criteria ensure that the variety is truly a new and consistent development. However, commercial profitability is not a requirement for registration. Even if a variety is not expected to be a major commercial success, it can still be protected if it satisfies the scientific and technical DUS standards.

NODIA

**237.** (A) Notified or existing local varieties
Under the PPV&FR Act, an "extant variety" includes those that have been notified under the Seeds Act of 1966, or are farmers' varieties, or are varieties about which there is common knowledge. The Act allows for the registration of these existing varieties to provide them with legal protection and to recognize the intellectual contributions of the communities that have maintained them. This is an important step toward safeguarding India's agricultural biodiversity.

**238.** (A) Community or individual compensation
Benefit sharing is a core principle of the PPV&FR Act, ensuring that communities or individuals who have contributed to the development of a new variety receive a fair share of the rewards. If a breeder uses a traditional variety or genetic resource to create a new, protected variety, they may be required to pay a portion of their profits into the National Gene Fund, which then compensates the original providers of the germplasm.

**239.** (A) Support for genetic conservation
The National Gene Fund was established under the PPV&FR Act to support the conservation and sustainable use of genetic resources. The fund is primarily financed through benefit-sharing payments from breeders and from the registration fees of extant varieties. It is used to reward farmers and communities for their roles in preserving landraces and wild relatives of crop plants, and to fund activities that promote the conservation of agricultural biodiversity across India.

safety cabinets and specialized ventilation systems to maintain negative pressure. BSL-3 ensures that both laboratory personnel and the surrounding community are protected from exposure to dangerous airborne pathogens.

**206.** (A) Sterility-based seed protection
Genetic Use Restriction Technologies, often called "terminator technology," were designed to produce plants that yield sterile seeds. This would prevent farmers from saving and replanting seeds from their harvest, forcing them to purchase new seeds every year. While developed for intellectual property protection, it faced significant ethical and social opposition and has not been commercialized, although the technical concepts remain a subject of biosafety discussion.

**207.** (A) Lack of specific mammalian receptors
Cry proteins from Bacillus thuringiensis are considered safe for mammals because they are highly specific to the gut environment of certain insects. Mammals lack the specific alkaline gut conditions and the unique receptors required for the Cry proteins to bind and form pores in the intestinal lining. Consequently, the ingested proteins are simply broken down as normal dietary protein in the acidic mammalian stomach, posing no toxic threat.

**208.** (B) It requires the demonstration of absolute zero risk for approval
Biosafety risk assessment is a systematic, case-by-case process that evaluates the potential hazards of a GMO to human health and the environment. It considers both intended and unintended effects and the biology of the host organism. However, it does not require a demonstration of absolute zero risk, as no activity is entirely without risk. Instead, it aims to ensure that the risks are managed and acceptable.

**209.** (A) Extensive wind or insect pollination
The risk of outcrossing, or the transfer of genes from a transgenic crop to other plants, is significantly increased in species that rely heavily on wind or insect pollination. These mechanisms can carry pollen over long distances, potentially reaching wild relatives or neighboring non-GM fields. Managing this risk involves establishing isolation distances and using biological containment methods to prevent the unintended spread of the transgene through the environment.

**210.** (C) A is true but R is false.
The Genetic Engineering Appraisal Committee is indeed the apex regulatory body for GMOs in India. However, it does not operate under the Ministry of Agriculture. Instead, it is housed within the Ministry of Environment, Forest and Climate Change. The GEAC is responsible for the final approval of large-scale field trials and the commercial release of all genetically modified organisms and products into the environment in India.

**211.** (C) Organization or institute level
The Institutional Biosafety Committee is the first level of regulatory oversight in the Indian biosafety framework. Every organization or institute involved in recombinant DNA research is mandated to constitute an IBSC. This committee is responsible for reviewing and monitoring all ongoing biotech projects at the local level, ensuring that they comply with the national safety guidelines and that proper containment measures are in place for the laboratory work.

**212.** (C) Research and field trial monitoring
The Review Committee on Genetic Manipulation is a national-level body in India that operates under the Department of Biotechnology. Its primary mandate is to monitor the safety of ongoing research projects and to grant approvals for small-scale, confined field trials of transgenic crops. The RCGM reviews the data generated from laboratory and greenhouse experiments to ensure that any potential environmental or health risks are properly assessed.

**213.** (C) MoEFCC (Environment Ministry)
The Genetic Engineering Appraisal Committee is the statutory body responsible for the regulation of GMOs in India. It is established under the Ministry of Environment, Forest and Climate Change. This placement reflects the committee's focus on assessing the environmental impact and safety of genetically modified organisms before they are approved for large-scale release or commercial use, ensuring that such activities do not harm the nation's biodiversity.

**214.** (A) Mandatory isolation distances
Confined field trials in India are subject to strict regulatory requirements to prevent

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**248.** (A) Obtain permission to access genetic resources
Prior Informed Consent is a core principle of the Nagoya Protocol, requiring researchers and companies to obtain the permission of the provider country before accessing its genetic resources. This process involves providing clear information about how the resources will be used and negotiating mutually agreed terms for the sharing of any benefits that might arise. PIC ensures that the rights and interests of the provider country and its indigenous communities are respected.

**249.** (A) 1992
The Convention on Biological Diversity was opened for signature at the United Nations Conference on Environment and Development, popularly known as the Earth Summit, held in Rio de Janeiro in 1992. This historic summit brought together world leaders to address global environmental challenges. The CBD was a major outcome, marking the first time that the conservation of biological diversity was recognized as a common concern of humankind and an integral part of sustainable development.

NODIA

**250.** (A) Organism with novel genetic combinations
Under the Cartagena Protocol, a Living Modified Organism is defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. This definition specifically targets organisms created through techniques like recombinant DNA technology or the fusion of cells beyond the taxonomic family. It distinguishes LMOs from plants or animals produced through traditional breeding and selection methods, which have long been used in agriculture.

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# CHAPTER 08
# Molecular Biology

2004

**1.** Which mechanism best describes the Wobble hypothesis regarding the genetic code?
(A) Relaxed complementary base pairing at the third position of the codon
(B) Strict Watson-Crick base pairing at all three codon positions
(C) Ribosomal shifting to an alternate reading frame during translation
(D) Immediate termination of protein synthesis upon encountering a rare codon

2014

**2.** How are Class I aminoacyl-tRNA synthetases structurally distinguished from Class II?
(A) They feature a β-sheet active site and attach amino acids specifically to the 3' OH.
(B) They exclusively recognize anticodon loops without interacting with the acceptor stem.
(C) They possess a Rossmann fold and attach the amino acid to the terminal 2' OH.
(D) They function solely as monomers in eukaryotic cells while Class II are hexameric.

2001

**3.** What is the correct composition of the prokaryotic 70S ribosome?
(A) 50S and 30S subunits containing 23S, 5S, and 16S rRNAs
(B) 40S and 30S subunits containing 18S and 5S rRNAs
(C) 60S and 40S subunits containing 28S, 5.8S, 5S, and 18S rRNAs
(D) 50S and 40S subunits containing 23S and 18S rRNAs

2010

**4.** Identify the correct statement regarding prokaryotic translation initiation.
(A) IF-1 binds the P site to prevent premature initiator tRNA entry.
(B) IF-3 actively hydrolyzes ATP to facilitate 50S subunit assembly.
(C) The Shine-Dalgarno sequence base-pairs with the 23S ribosomal RNA.
(D) IF-2 is a GTPase that facilitates fMet-tRNAfMet binding to the P site.

2015

**5.** Identify the INCORRECT statement regarding eukaryotic translation initiation.
(A) The Kozak sequence facilitates start codon recognition.
(B) The initiator tRNA binds directly to the A site before scanning begins.
(C) The 40S subunit scans the mRNA from the 5' cap.
(D) eIF4G directly binds the 3' poly-A tail, circularizing the mRNA.

2005

**6.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): During elongation, EF-Tu prevents premature peptide bond formation until correct codon-anticodon pairing occurs.
Reason (R): EF-Tu physically masks the amino acid linked to the tRNA, only releasing it upon GTP hydrolysis after correct A-site accommodation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**7.** Which of the following is NOT involved in prokaryotic translation termination?
(A) $RF_1$
(B) RF2
(C) RF3
(D) $eRF_1$

2010

**8.** What is the most appropriate description of the mode of action of Puromycin?
(A) It mimics aminoacyl-tRNA and causes termination by entering the A site.
(B) It binds to the 30S ribosomal subunit to inhibit decoding accuracy.
(C) It cleaves the 23S rRNA at the peptidyl transferase center directly.

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(D) It inhibits translocation by permanently locking EF-G to the ribosome.

2011

**9.** Match List I with List II.

List I: (Chaperones)

P. Hsp70

Q. GroEL

R. GroES

S. Ubiquitin

List II: (Characteristics)

1. Double-ring chaperonin complex
2. Co-chaperone lid structure
3. Marks proteins for proteasomal degradation
4. Binds nascent hydrophobic peptides co-translationally

(A) P-1, Q-4, R-3, S-2

(B) P-4, Q-1, R-2, S-3

(C) P-4, Q-2, R-1, S-3

(D) P-2, Q-1, R-4, S-3

2011

**10.** Given below are certain statements about protein N-glycosylation.

I. It occurs predominantly on asparagine residues.

II. It requires the consensus sequence Asn-X-Ser/Thr.

III. The oligosaccharide is assembled directly on the target protein in the Golgi.

Which of the above statement(s) is/are correct?

(A) II and III

(B) I and II

(C) I and III

(D) I

2025

**11.** Which event directly causes the release of the signal recognition particle (SRP) from its receptor?

(A) GTP hydrolysis by both the SRP and the SRP receptor

(B) Binding of the ribosome to the translocon

(C) Cleavage of the signal peptide by signal peptidase

(D) ATP binding to the Sec61 complex

2012

**12.** Which classification applies to the lac operon's mode of regulation?

(A) Strictly positive repressible

(B) Negative repressible and positive inducible

(C) Negative inducible and positive inducible

(D) Strictly negative repressible

2013

**13.** Compared to standard repressor control, what unique regulatory feature does the trp operon attenuation mechanism employ?

(A) Allosteric inhibition of RNA polymerase

(B) Cleavage of the repressor protein by proteases

(C) Post-translational modification of tryptophan

(D) Coupling of transcription and translation dynamics

2013

**14.** Which is the LEAST RELEVANT domain for direct sequence-specific DNA binding in eukaryotic transcription factors?

(A) Zinc finger

(B) Helix-turn-helix

(C) SH2 domain

(D) Leucine zipper

2004

**15.** What inference can be made when a promoter region exhibits heavy cytosine methylation and histone deacetylation?

(A) The gene is actively undergoing splicing.

(B) The gene is transcriptionally repressed.

(C) The chromatin is in a euchromatic state.

(D) The transcript will bypass polyadenylation.

2008

**16.** Identify the primary enzyme responsible for the 5' capping of eukaryotic mRNA.

(A) Poly(A) polymerase

(B) Endonuclease

(C) Terminal deoxynucleotidyl transferase

(D) Guanylyltransferase

2014

**17.** What is the primary advantage of alternative splicing in eukaryotic organisms?

(A) It increases proteome diversity from a limited number of genes.

(B) It eliminates the requirement for post-translational modifications.

(C) It ensures the complete degradation of all mutated transcripts.

(D) It blocks the action of viral reverse transcriptase in the host.

2010

**18.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).

Assertion (A): Adenosine-to-inosine (A-to-I) RNA editing can alter the amino acid sequence of the encoded protein.

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**240.** (C) III → II → IV → I
The registration of a new variety under the PPV&FR Act follows a clear sequence: first, the application is filed; second, the variety undergoes DUS testing to verify its characteristics; third, the application is advertised in the Plant Variety Journal to allow for any public objections; and finally, if all requirements are met, a Certificate of Registration is issued. This process ensures that all protected varieties are truly distinct, uniform, and stable.

**241.** (B) The elimination of all agricultural biotechnology
The Convention on Biological Diversity is an international treaty with three primary goals: the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from the use of genetic resources. It does not aim to eliminate biotechnology; rather, it recognizes its potential for human welfare while emphasizing the need for its safe and equitable application to protect the world's diverse biological heritage.

**242.** (A) Access and Benefit Sharing (ABS)
The Nagoya Protocol is a landmark supplementary agreement to the CBD that focuses on Access and Benefit Sharing. It provides a transparent legal framework for the effective implementation of one of the CBD's three objectives: the fair and equitable sharing of benefits arising from the utilization of genetic resources. By setting out clear rules for accessing these resources and sharing the rewards, the protocol supports both biodiversity conservation and sustainable development.

**243.** (A) Safe handling and use of LMOs
The Cartagena Protocol on Biosafety is an international agreement that aims to ensure the safe handling, transport, and use of living modified organisms resulting from modern biotechnology. Its primary focus is on preventing any adverse effects of LMOs on the conservation and sustainable use of biological diversity, while also considering risks to human health. It provides a framework for countries to make informed decisions about importing LMOs into their environment.

NODIA

**244.** (B) Advanced Informed Agreement (AIA) procedure
The Advance Informed Agreement (AIA) procedure is a key mechanism of the Cartagena Protocol. It applies to the first intentional transboundary movement of an LMO intended for introduction into the environment of the importing country. Under this procedure, the exporter must provide detailed information about the LMO, and the importer must give explicit consent based on a thorough risk assessment before the movement can occur, ensuring transparency and safety in international trade.

**245.** (B) Biosafety Clearing-House (BCH)
The Biosafety Clearing-House is an international online platform established under the Cartagena Protocol to facilitate the exchange of scientific, technical, environmental, and legal information on LMOs. It serves as a centralized database where member countries can access data on national biosafety regulations, risk assessment summaries, and decisions regarding the import or release of LMOs. The BCH is essential for ensuring transparency and informed decision-making among all parties to the protocol.

**246.** (A) LMOs in transit or intended for contained use
While the Cartagena Protocol's AIA procedure is designed for LMOs intended for environmental release, certain categories are explicitly excluded. This includes LMOs in transit through a country or those intended for contained use, such as in a laboratory or greenhouse. Additionally, LMOs intended for direct use as food, feed, or for processing are subject to a different, simplified procedure, reflecting their lower risk of becoming established in the natural environment.

**247.** (A) 2010
The Nagoya Protocol was formally adopted in 2010 during the tenth meeting of the Conference of the Parties to the CBD held in Nagoya, Japan. It represents a major step forward in international environmental law, providing a concrete mechanism for sharing the benefits from the use of genetic resources and traditional knowledge. The protocol entered into force in 2014, and its implementation is a key priority for many nations seeking to protect their biodiversity.

(B) RF3 - Recognizes the UAA codon directly in prokaryotes
(C) EF-G - Facilitates translocation in prokaryotes
(D) eIF4E - Binds the 5' cap of eukaryotic mRNA

2013

**27.** What is the most appropriate role for the proteasome in eukaryotic cells?
(A) Degrading ubiquitinated misfolded or short-lived proteins
(B) Synthesizing specific chaperonin complexes
(C) Folding nascent polypeptides emerging from the ribosome
(D) Cleaving signal sequences from secretory proteins

2019

**28.** How does the cAMP-CAP complex affect the lac operon?
(A) It physically blocks RNA polymerase binding.
(B) It degrades lactose into glucose and galactose.
(C) It bends the DNA near the promoter to enhance RNA polymerase recruitment.
(D) It binds directly to the lac repressor, altering its conformation.

2015

**29.** Which is the LEAST RELEVANT factor in the formation of the Trp operon terminator hairpin?
(A) High levels of charged tRNATrp
(B) Rapid translation of the leader peptide
(C) Pairing of regions 3 and 4 in the leader mRNA
(D) Binding of the Trp repressor to the operator

2002

**30.** Given below are certain statements about epigenetic regulation.
I. DNA methylation usually correlates with gene silencing.
II. Histone acetylation neutralizes the positive charge of lysine residues.
III. ATP-dependent chromatin remodeling complexes statically lock nucleosomes.
IV. Histone modifications exclusively occur on the globular core domains.
V. The "histone code" hypothesis dictates that modifications create binding sites for effector proteins.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) II, IV and V
(C) I, II and V
(D) I, III and IV

2007

**31.** What inference can be made regarding Group I introns?
(A) They require a spliceosome for catalytic activity.
(B) They use a free guanosine cofactor to initiate self-splicing.
(C) They generate a lariat structure during the splicing reaction.
(D) They are primarily found in eukaryotic mRNA encoding structural proteins.

2021

**32.** Which EXCEPTION exists in the typical comparison between siRNAs and miRNAs?
(A) siRNAs usually match their targets perfectly, whereas miRNAs often have mismatches.
(B) siRNAs typically mediate mRNA cleavage, whereas miRNAs frequently cause translational repression.
(C) Both utilize the RISC complex, but their origin pathways differ.
(D) siRNAs are endogenous to all plants, whereas miRNAs are strictly introduced by viruses.

2015

**33.** Determine the role of Dicer in RNA interference.
(A) It cleaves long double-stranded RNA into short 21-23 nucleotide fragments.
(B) It transcribes double-stranded RNA into mRNA sequences for later translation.
(C) It degrades single-stranded RNA molecules exclusively within the cell nucleus.
(D) It adds a poly-A tail to small interfering RNAs to ensure cytoplasmic stability.

2015

**34.** What is the primary advantage of utilizing the nicotianamine synthase gene in genetic biofortification?
(A) It increases β-carotene levels in endosperm.
(B) It confers resistance against lepidopteran pests.
(C) It facilitates iron and zinc chelation and transport within the plant.
(D) It completely halts the synthesis of phytic acid.

2023

**35.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Gene pyramiding is more durable

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from methionine.

2003

**54.** Identify the correct statement regarding O-linked glycosylation.
(A) It occurs strictly in the endoplasmic reticulum.
(B) It involves a dolichol phosphate lipid anchor.
(C) It targets the hydroxyl oxygen of serine or threonine residues.
(D) It is characterized by the attachment of an en bloc 14-sugar oligosaccharide.

2013

**55.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The ubiquitin-proteasome pathway requires ATP hydrolysis.
Reason (R): ATP is required by ubiquitin-activating enzyme (E1) to form a high-energy thioester bond with ubiquitin.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**56.** Which is an EXCEPTION to the classical secretory pathway transport?
(A) Nuclear localized transcription factors
(B) Soluble lysosomal enzymes
(C) Transmembrane plasma membrane proteins
(D) Extracellular matrix proteins

2021

**57.** Identify the INCORRECT statement regarding regulatory element and its function in the lac operon.
(A) LacI - Repressor protein
(B) LacA - Lactose permease
(C) LacO - Operator DNA sequence
(D) LacZ - B-galactosidase

2002

**58.** What causes the formation of the antiterminator (regions 2-3 pairing) in the Trp operon?
(A) Plentiful tryptophan levels causing rapid ribosome movement over the leader peptide
(B) Ribosome stalling at consecutive Trp codons in the leader sequence
(C) Binding of the Trp repressor to region 4
(D) Complete absence of RNA polymerase

NODIA

2013

**59.** Which is the most relevant characteristic of chromatin containing heavily acetylated histones?
(A) Transcriptional activation
(B) Highly condensed structure
(C) Resistance to DNase I digestion
(D) Centromeric localization

2023

**60.** Given below are certain statements about eukaryotic mRNA 3' polyadenylation.
I. It requires the AAUAAA consensus sequence.
II. It is synthesized by RNA polymerase II from a poly-T DNA template.
III. It enhances mRNA stability and translational efficiency.
IV. Poly(A) polymerase performs the addition without a DNA template.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) II, III and IV
(C) I, III and IV
(D) I and III

2016

**61.** What inference can be made regarding a pre-mRNA lacking snRNP U1 binding capability?
(A) It will undergo enhanced self-splicing.
(B) It will be immediately translated into a functional protein.
(C) The 3' poly-A tail will be elongated indefinitely.
(D) The 5' splice site will not be properly recognized.

2011

**62.** Which definition correctly applies to RNA editing?
(A) Splicing together of exons from completely different pre-mRNAs
(B) Addition of non-templated nucleotides solely at the 3' end
(C) Post-transcriptional alteration of the nucleotide sequence of an RNA transcript
(D) Degradation of mRNA by microRNAs

2005

**63.** How do siRNAs primarily cause post-transcriptional gene silencing?
(A) By directly binding to the promoter and blocking RNA polymerase
(B) By guiding the RISC complex to cleave perfectly complementary target mRNA
(C) By methylating the target gene's DNA
(D) By preventing the export of mRNA from the

Q. Leucine Zipper
R. Helix-Turn-Helix
S. Homeodomain

List II: (Description)

1. Contains highly conserved 60 amino acids typical of Hox genes
2. Interdigitating hydrophobic residues facilitating dimerization
3. Relies on a coordinated metal ion for structural stability
4. Found prominently in the bacterial Lac repressor

(A) P-2, Q-3, R-1, S-4
(B) P-3, Q-1, R-4, S-2
(C) P-1, Q-4, R-2, S-3
(D) P-3, Q-2, R-4, S-1

2005

**46.** What defines an enhancer element in eukaryotic gene regulation?
(A) A sequence that positions RNA polymerase II at the transcription start site.
(B) A trans-acting protein factor that binds tightly to the RNA polymerase.
(C) A cis-acting DNA sequence that stimulates transcription from a distance.
(D) A regulatory RNA molecule that stabilizes the mRNA against degradation.

2012

**47.** Which statement accurately captures the concept application of using the GUS reporter system in plant biotechnology?
(A) It allows continuous non-destructive imaging of living cells.
(B) It requires the substrate X-Gluc, producing a blue precipitate in fixed tissues.
(C) It necessitates the application of luciferin to emit photons.
(D) It functions exclusively in prokaryotic expression vectors.

2007

**48.** What is the most appropriate explanation for the degenerate nature of the genetic code?
(A) Multiple amino acids can be coded by a single codon.
(B) Codons naturally degrade over cellular lifespan.
(C) The code overlaps within a single open reading frame.
(D) A single amino acid can be encoded by more than one synonymous codon.

2015

**49.** Which mechanism describes the proofreading ability of aminoacyl-tRNA synthetases?
(A) A distinct editing domain that hydrolyzes mischarged amino acids from the tRNA
(B) Ribosomal ejection of improperly folded tRNAs
(C) Cleavage of the mRNA transcript upon stalling
(D) Exonucleolytic degradation of the anticodon loop

2006

**50.** Given below are certain statements concerning the 80S eukaryotic ribosome.
I. It contains a 28S rRNA molecule in its large subunit.
II. It initiates translation exclusively through Shine-Dalgarno sequences.
III. Its assembly requires the nucleolus.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I

2017

**51.** What is the role of eIF4G during translation?
(A) It acts as a scaffold linking the 5' cap complex to the 40S subunit.
(B) It provides the GTP required for large subunit joining.
(C) It cleaves the mRNA after translation termination.
(D) It catalyzes the formation of the first peptide bond.

2006

**52.** What is the consequence of inhibiting the 23S rRNA peptidyl transferase center?
(A) Prevention of transcription initiation
(B) Failure of the ribosome to recognize the start codon
(C) Inability to aminoacylate tRNA
(D) Cessation of translation elongation due to lack of peptide bond formation

2024

**53.** Which factual detail defines the function of $RF_1$ in Escherichia coli?
(A) It recognizes the stop codons UAA and UGA.
(B) It recognizes the stop codons UAA and UAG.
(C) It binds specifically to the 5' cap.
(D) It catalyzes the removal of formyl groups

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2016

**72.** Which classification best fits the enzyme peptidyl transferase?
(A) A ribozyme integral to the large ribosomal subunit
(B) A classic protein-based ligase
(C) An RNA-directed DNA polymerase
(D) A cytosolic ATP-dependent kinase

2002

**73.** Which of the following describes the mechanism of eukaryotic initiation factor eIF2?
(A) It binds mRNA to the 40S subunit.
(B) It delivers the initiator Met-tRNAi to the 40S subunit in a GTP-dependent manner.
(C) It forms the peptide bond between the first two amino acids.
(D) It strictly recognizes the Kozak sequence.

2012

**74.** What is the primary position of ribosome recycling in bacteria post-termination?
(A) Binding of $IF_1$ to the empty ribosome
(B) Action of the Ribosome Recycling Factor (RRF) alongside EF-G and GTP hydrolysis
(C) Cleavage of the ribosomal RNA
(D) Attachment of the SRP complex

2007

**75.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Diphtheria toxin inhibits eukaryotic translation.
Reason (R): It catalyzes the ADP-ribosylation of eEF2, blocking translocation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**76.** Identify the INCORRECT statement about protein folding chaperones.
(A) They ensure proteins fold into their minimum-energy native states.
(B) They bind to exposed hydrophobic regions of unfolded proteins.
(C) They permanently integrate into the final structure of the folded protein.
(D) Their cycle often involves ATP binding and hydrolysis.

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2001

**77.** Which post-translational modification heavily influences transcription factor activity by adding negative charges?
(A) Methylation
(B) Farnesylation
(C) Ubiquitination
(D) Phosphorylation

2015

**78.** What determines whether a newly synthesized protein enters the secretory pathway?
(A) The presence of a poly-A tail on its mRNA
(B) A highly basic nuclear localization signal
(C) An N-terminal hydrophobic signal peptide
(D) The phosphorylation of its C-terminus

2005

**79.** In the context of the lac operon, what defines the role of IPTG?
(A) It activates the cAMP-CAP complex directly.
(B) It is a gratuitous inducer that binds the repressor but is not metabolized.
(C) It cleaves lactose into its monosaccharides.
(D) It functions as a co-repressor.

2016

**80.** Select the correct order of elements in a typical eukaryotic gene promoter.
(A) Enhancer → TATA Box → Transcription Start Site → Translation Start Codon
(B) TATA Box → Enhancer → Translation Start Codon → Transcription Start Site
(C) Transcription Start Site → TATA Box → Enhancer → Translation Start Codon
(D) Enhancer → Translation Start Codon → TATA Box → Transcription Start Site

2010

**81.** Which regulatory mechanism employs a terminator/antiterminator stem-loop structure?
(A) Trp operon attenuation
(B) Lac operon catabolite repression
(C) Eukaryotic RNA interference
(D) Group II intron splicing

2013

**82.** Which property is LEAST RELEVANT to the function of eukaryotic enhancers?
(A) They are completely position-independent relative to the promoter.
(B) They can function in either orientation.
(C) They are transcribed into the final coding region of the mRNA.
(D) They serve as binding sites for transcriptional

nucleus

2015

**64.** When considering metagenomic bioprospecting, which strategy is most appropriate for identifying a novel enzyme without prior sequence knowledge?
(A) Sequence-driven screening using specific PCR primers
(B) Amplification of strictly the 16S rRNA gene
(C) Traditional selective plating and culturing techniques
(D) Function-driven screening of expression libraries for specific enzymatic activity

2016

**65.** Match List I with List II.
List I: (Biofortification Target)
P. Provitamin A
Q. Iron
R. Zinc
S. Essential amino acids
List II: (Metabolic Component)
1. Nicotianamine synthase
2. Phytoene synthase
3. Ferritin
4. Amaranth seed albumin (AmA1)
(A) P-2, Q-3, R-1, S-4
(B) P-3, Q-2, R-1, S-4
(C) P-2, Q-1, R-3, S-4
(D) P-1, Q-4, R-2, S-3

2006

**66.** Identify the factual advantage of a marker-assisted gene pyramiding approach over conventional phenotypic selection.
(A) It eliminates the biological requirement to grow the plants in soil.
(B) It directly alters the primary DNA sequence without using crossing.
(C) It allows simultaneous selection of multiple genes with similar traits.
(D) It restricts genomic recombination strictly to the telomeric regions.

2003

**67.** Which of the following is a disadvantage of using the GFP reporter system?
(A) It can sometimes aggregate or cause steric hindrance when fused to certain target proteins.
(B) It requires the destruction of the plant tissue for visualization.
(C) It depends entirely on the addition of an exogenous substrate.
(D) It is fundamentally incompatible with eukaryotic cells.

2001

**68.** Which structure defines a hairpin ribozyme?
(A) A massive complex of RNA and over 50 proteins
(B) A perfectly straight, rigid double helix of 150 base pairs
(C) A single, large unstructured RNA loop
(D) Two independently folding domains (loops A and B) that dock to form the active site

2016

**69.** Which statement concerning codon usage bias is correct?
(A) All synonymous codons are utilized with equal frequency by all organisms.
(B) Organisms prefer specific synonymous codons matching tRNA abundances.
(C) Bias occurs strictly and exclusively at the first position of the codon.
(D) Viral genomes universally adopt host codon usage patterns without exception.

2018

**70.** Given below are certain statements about tRNA cloverleaf structure.
I. The D loop contains dihydrouridine residues.
II. The TΨC loop contains pseudouridine.
III. The anticodon loop interacts directly with the amino acid.
IV. The acceptor stem always terminates in a 5'-CCA-3' sequence.
V. Base pairing between specific regions generates the tertiary L-shape.
Which of the above statement(s) is/are correct?
(A) II, III, IV and V
(B) I, II, IV and V
(C) I, II and V
(D) I, III and V

2012

**71.** Which of the following identifies the correct ribosome site and its distinct function during elongation?
(A) A site - Holds the discharged tRNA prior to exit
(B) E site - Accepts the incoming aminoacyl-tRNA
(C) A site - Primarily binds mRNA only
(D) P site - Anchors the growing peptidyl-tRNA chain

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against pathogen resistance breakdown than single-gene deployment.
Reason (R): Pathogens must simultaneously undergo multiple specific mutations to overcome multiple stacked resistance genes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2013

**36.** Which structural domain describes a motif where two α helices are separated by a short non-helical loop, commonly functioning in protein dimerization?
(A) Zinc finger
(B) Helix-turn-helix
(C) B-barrel
(D) Helix-loop-helix

2023

**37.** Identify the factual component required for the autocatalytic activity of Group II introns.
(A) An external guanosine nucleophile
(B) A specific internal adenosine forming a 2'-5' phosphodiester bond
(C) ATP hydrolysis by the spliceosome
(D) Drosha-mediated primary transcript cleavage

2018

**38.** Which process requires the enzyme Drosha?
(A) Processing of pre-miRNA into mature miRNA in the cytoplasm
(B) Splicing of pre-mRNA to mature mRNA
(C) Cleavage of pri-miRNA to pre-miRNA in the nucleus
(D) Addition of the 5' 7-methylguanosine cap

2011

**39.** What is the cause of the cytidine-to-uridine (C-to-U) RNA editing found in specific plant organelles?
(A) Transamination by aminotransferase enzymes
(B) Excision of cytidine and insertion of uridine by RNA polymerase
(C) Methylation of the cytosine ring
(D) Deamination by specific cytidine deaminase enzymes

2014

**40.** Select the correct sequence for protein secretion in a eukaryotic cell.
(A) ER → Golgi → Secretory Vesicle → Plasma Membrane
(B) Golgi → ER → Endosome → Plasma Membrane
(C) ER → Lysosome → Golgi → Plasma Membrane
(D) Nucleus → ER → Secretory Vesicle → Golgi

2024

**41.** Which classification correctly identifies the Hepatitis Δ Virus (HDV) ribozyme?
(A) Self-splicing intron
(B) Small catalytic RNA involved in RNA genome cleavage and ligation
(C) Nucleolytic ribozyme requiring no metal ions for basic structural folding
(D) Spliceosomal RNA component

2019

**42.** When comparing wild relatives to cultivated crops for bioprospecting, what is typically the greatest advantage of wild relatives?
(A) Higher harvest index
(B) Presence of highly diverse allelic variations for stress tolerance
(C) Readily available reference genomes
(D) Complete absence of secondary metabolites

2006

**43.** Which pair regarding affinity-tag fusion systems is correct?
(A) His-tag - Purified using immobilized metal affinity chromatography (IMAC)
(B) GST-tag - Purified using immobilized nickel affinity chromatography
(C) Maltose-Binding Protein - Purified using chitin beads
(D) FLAG-tag - Purified using glutathione agarose

2014

**44.** Identify the INCORRECT statement regarding post-translational lipid modifications.
(A) Myristoylation generally occurs on an N-terminal glycine.
(B) Prenylation usually targets a C-terminal CaaX motif.
(C) Palmitoylation irreversibly attaches a lipid strictly via an amide linkage.
(D) Farnesylation involves the attachment of a 15-carbon isoprenoid group.

2009

**45.** Match List I with List II.
List I: (Transcription Factor Domain)
P. Zinc Finger

NODIA

activator proteins.

2021

**83.** What inference can be drawn from the observation of a specific genomic region possessing a highly methylated CpG island?
(A) The associated gene is likely transcriptionally silenced.
(B) The region actively recruits RNA polymerase II.
(C) The region is a hotspot for alternative splicing.
(D) The gene represents an actively transposing element.

2013

**84.** Which step is an EXCEPTION in the sequential addition of the eukaryotic 5' cap?
(A) Removal of the terminal phosphate of the pre-mRNA by RNA triphosphatase
(B) Addition of GMP by guanylyltransferase
(C) Methylation of the terminal guanine at the N7 position
(D) Splicing of the first intron to supply the cap structure

2021

**85.** Match List I with List II.
List I: (Splicing Component/Mechanism)
P. U1 snRNP
Q. U2 snRNP
R. Group I intron
S. Group II intron
List II: (Characteristic)
1. Binds to the branch point sequence
2. Uses an internal adenosine nucleophile
3. Uses an external guanosine nucleophile
4. Binds to the 5' splice site
(A) P-1, Q-4, R-2, S-3
(B) P-4, Q-1, R-3, S-2
(C) P-4, Q-2, R-1, S-3
(D) P-3, Q-1, R-4, S-2

2006

**86.** Which definition correctly outlines RNA editing via adenosine-to-inosine modification?
(A) It involves the direct replacement of purines with pyrimidines.
(B) It results strictly in frameshift mutations.
(C) It involves ADAR enzymes acting on double-stranded RNA regions.
(D) It bypasses the requirement for base-pairing rules during translation.

2006

**87.** Identify the correct pair linking the RNAi component to its specific action.
(A) Drosha - Cleaves dsRNA in the cytoplasm
(B) Dicer - Synthesizes pri-miRNA
(C) Exportin 5 - Transcribes shRNA constructs
(D) Argonaute - Cleaves the target mRNA within the RISC complex

2019

**88.** What is the most appropriate rationale for utilizing wild relatives in plant bioprospecting?
(A) They possess traits like drought resistance lost during crop domestication.
(B) They lack complex biochemical pathways, making gene isolation easier.
(C) Their genomes are significantly smaller and always diploid.
(D) They naturally express high levels of transgenic reporter genes.

2003

**89.** Which mechanism describes the fundamental approach to genetic biofortification for β-carotene in rice endosperm?
(A) Deletion of repressor genes governing intrinsic rice β-carotene pathways.
(B) Introduction of genes bridging the gap from GGPP to β-carotene.
(C) Selective breeding of naturally deep-yellow rice varieties exclusively.
(D) Soaking rice seeds in carotene-rich bacterial cultures for enrichment.

NODIA

2001

**90.** Given below are certain statements regarding gene pyramiding.
I. It is generally achieved by repeated backcrossing and marker-assisted selection.
II. It reduces the selection pressure on pathogens compared to single-gene deployment.
III. It involves the integration of only identical alleles at the same locus.
Which of the above statement(s) is/are correct?
(A) II and III
(B) I and II
(C) I and III
(D) I

2018

**91.** Identify the INCORRECT statement about affinity-tag fusion systems.
(A) They alter the fundamental genetic code to incorporate non-natural amino acids.
(B) They greatly simplify the downstream purification of recombinant proteins.

(C) The tag is always covalently linked to the target protein until specifically cleaved.
(D) Protease cleavage sites are often engineered between the tag and the protein.

2006

**92.** Which factual attribute applies to the hairpin ribozyme?
(A) It requires a continuous stretch of 200 base pairs to function.
(B) It degrades cellular DNA during apoptosis.
(C) It catalyzes a reversible transesterification reaction yielding a 2',3'-cyclic phosphate and a 5'-hydroxyl.
(D) It functions exclusively within the spliceosome.

2019

**93.** What defines the specific parameter of codon usage bias in molecular biology?
(A) The exclusion of specific amino acids from certain protein structures
(B) The mandatory usage of a single codon for each amino acid
(C) The failure of the ribosome to decode rare codons completely
(D) The non-random usage of synonymous codons during translation

2001

**94.** Which characteristic firmly classifies an enzyme as a Class I aminoacyl-tRNA synthetase?
(A) Binding to the major groove of the tRNA acceptor stem
(B) Consistently existing only as a tetrameric protein
(C) Binding to the minor groove of the tRNA acceptor stem and utilizing a Rossmann fold
(D) Requiring no ATP for amino acid activation

2001

**95.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The 16S rRNA plays a critical role in prokaryotic translation initiation.
Reason (R): Its 3' end contains an anti-Shine-Dalgarno sequence that base-pairs with the mRNA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**96.** Which is an advantage of understanding the Kozak consensus sequence?
(A) It allows researchers to optimize mRNA transcripts for maximal translation in prokaryotes.
(B) It directs the specific degradation of viral RNA.
(C) It provides a binding site for release factors.
(D) It allows the design of expression vectors that maximize translation efficiency in eukaryotic systems.

2022

**97.** Identify the correct process describing how $eEF_1A$ contributes to eukaryotic translation.
(A) It escorts the aminoacyl-tRNA to the ribosomal A-site in a GTP-dependent manner.
(B) It catalyzes the formation of the peptide bond.
(C) It facilitates the translocation of the ribosome along the mRNA.
(D) It mediates the release of the nascent polypeptide chain.

2008

**98.** Identify the functional analog of prokaryotic EF-G in eukaryotes.
(A) $eEF_1A$
(B) $eEF_1B$
(C) eEF2
(D) eIF3

2024

**99.** What causes the ultimate disassembly of the prokaryotic ribosome post-translation?
(A) The coordinate action of RRF, EF-G, and IF3
(B) The depletion of cellular ATP
(C) Cleavage of the mRNA by exonucleases
(D) Spontaneous dissociation upon encountering a stop codon

2013

**100.** Given below are certain statements about translation inhibitors.
I. Streptomycin causes misreading of the genetic code in prokaryotes.
II. Cycloheximide inhibits peptidyl transferase activity in eukaryotic 60S subunits.
III. Chloramphenicol broadly blocks eukaryotic translation.
IV. Puromycin functions strictly as a competitive inhibitor of RNA polymerase.
Which of the above statement(s) is/are correct?

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(A) II and III
(B) I, III and IV
(C) I, II and IV
(D) I and II

2008

**101.** Which structural form does the ubiquitin-proteasome system directly target for degradation?
(A) Actively translating mRNA
(B) Polyubiquitinated proteins
(C) Properly folded native proteins
(D) Unmodified histones

2023

**102.** What defines the primary distinction between N-linked and O-linked glycosylation?
(A) N-linked occurs in the cytoplasm; O-linked occurs in the nucleus.
(B) N-linked utilizes strictly glucose; O-linked utilizes strictly mannose.
(C) N-linked occurs on Asparagine; O-linked occurs on Serine/Threonine.
(D) N-linked does not require lipid carriers, while O-linked requires dolichol.

2009

**103.** Which is an EXCEPTION to the modifications occurring within the Golgi apparatus?
(A) O-linked glycosylation
(B) Modification and pruning of N-linked oligosaccharides
(C) Sorting of proteins to secretory vesicles
(D) Addition of initial en bloc oligosaccharide to Asparagine

2013

**104.** Which concept application describes the primary use of IPTG in molecular cloning?
(A) Inducing recombinant protein expression under the control of the lac promoter
(B) Serving as a carbon source for bacterial growth
(C) Staining bacterial colonies blue
(D) Inhibiting bacterial cell wall synthesis

2021

**105.** Match List I with List II.
List I: (Eukaryotic Regulatory Element/Factor)
P. Silencer
Q. Promoter
R. General Transcription Factors (GTFs)
S. Specific Transcription Factors
List II: (Primary Characteristic)
1. Bind to enhancers to modulate transcription rates
2. Required for basal transcription initiation at the core promoter
3. Binds repressor proteins to downregulate transcription
4. Contains the TATA box and initiator elements
(A) P-4, Q-3, R-1, S-2
(B) P-3, Q-4, R-2, S-1
(C) P-3, Q-2, R-4, S-1
(D) P-1, Q-4, R-2, S-3

2005

**106.** Identify the factual outcome of heavy DNA methylation at a gene promoter.
(A) Immediate activation of DNA polymerase
(B) Steric hindrance of transcription factor binding and recruitment of histone deacetylases
(C) Enhanced binding of RNA polymerase III
(D) Increased rate of alternative splicing

NODIA

2012

**107.** What is the most appropriate functional description of the 5' mRNA cap?
(A) It protects the mRNA from 5'-to-3' exonucleases and assists in ribosome binding.
(B) It dictates the site of polyadenylation.
(C) It codes for the initial methionine residue.
(D) It functions as a ribozyme to excise the first intron.

2023

**108.** Which mechanism characterizes the action of a spliceosome?
(A) It physically cleaves DNA to remove introns prior to transcription.
(B) It synthesizes a complementary RNA strand to bridge exons.
(C) It utilizes snRNAs to recognize splice sites and catalyze two transesterification reactions.
(D) It heavily methylates intron sequences to suppress their translation.

2015

**109.** Which sequence accurately describes the production of mature miRNA?
(A) Pri-miRNA → Pre-miRNA → Mature miRNA
(B) Pre-miRNA → Pri-miRNA → Mature miRNA
(C) Mature miRNA → Pre-miRNA → Pri-miRNA
(D) Pri-miRNA → Mature miRNA → Pre-miRNA

2004

**110.** Given below are certain statements regarding post-transcriptional gene silencing.

I. It requires sequence homology between the small RNA and the target mRNA.
II. It strictly operates by modifying chromosomal DNA structure.
III. It involves the Argonaute protein family.
IV. It can be systematically spread throughout certain plants via plasmodesmata.
V. It completely bypasses the need for the RISC complex.

Which of the above statement(s) is/are correct?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I, IV and V

2008

**111.** Identify the INCORRECT statement about biofortification using transgenic approaches.
(A) It targets the enhancement of specific micronutrients in edible plant parts.
(B) It is strictly limited to traits already present within the crop's natural gene pool.
(C) It often utilizes tissue-specific promoters to direct accumulation.
(D) The Golden Rice project is a classic example of this methodology.

2022

**112.** What inference can be made if a gene pyramiding strategy successfully stacks three different Bt toxin genes in cotton?
(A) The plant will become highly susceptible to herbicides.
(B) The plant will cease all natural defense mechanisms.
(C) The transcription of endogenous genes will be permanently silenced.
(D) The probability of insects developing resistance to all three toxins simultaneously is mathematically extremely low.

2022

**113.** Which cause and effect relationship correctly defines a functional gene fusion?
(A) Fusing a GFP tag to a protein allows for its visual tracking within a living cell.
(B) Fusing a signal peptide sequence causes the protein to be retained in the cytoplasm.
(C) Fusing a GST tag ensures the immediate degradation of the protein.
(D) Fusing a promoter directly to another promoter yields a functional hybrid protein.

2007

**114.** Which definition precisely describes a ribozyme?
(A) An enzyme that specifically degrades ribosomes
(B) A structural protein holding the ribosomal subunits together
(C) An RNA molecule capable of acting as an enzyme to catalyze specific biochemical reactions
(D) A DNA sequence that codes for ribosomal RNA

2018

**115.** Identify the correct pair connecting the RNA component to its defining structural motif.
(A) tRNA - Cloverleaf and L-shape
(B) rRNA - Zinc finger
(C) tRNA - Helix-turn-helix
(D) snoRNA - Lariat structure

2013

**116.** Which point describes the primary advantage of the wobble position?
(A) It strictly enforces translation fidelity by excluding non-cognate tRNAs.
(B) It causes intentional mutations in the synthesized protein to increase diversity.
(C) It prevents the binding of release factors until the stop codon is encountered.
(D) It allows a single tRNA to recognize multiple synonymous codons, reducing tRNA requirements.

2013

**117.** What distinguishes eukaryotic 80S ribosomes from prokaryotic 70S ribosomes in terms of their core enzymatic activity?
(A) Prokaryotes use RNA for peptidyl transfer, while eukaryotes use ribosomal proteins.
(B) Both utilize RNA for peptidyl transfer, but the catalytic rRNA is 23S in prokaryotes and 28S in eukaryotes.
(C) Eukaryotic ribosomes completely lack a P-site.
(D) Prokaryotic ribosomes assemble in the nucleolus.

2012

**118.** Which concept application justifies the use of Kozak sequence consensus optimization in synthetic biology?
(A) Improving the termination efficiency of RNA polymerase
(B) Increasing the degradation rate of recombinant proteins
(C) Enhancing the efficiency of translational

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start site recognition in eukaryotic expression systems
(D) Directing the synthesized protein specifically to the mitochondrion

2025

**119.** Determine the role of IF-3 in prokaryotic initiation.
(A) It forms the peptide bond.
(B) It adds a formyl group to methionine.
(C) It shifts the ribosome exactly three nucleotides forward.
(D) It binds to the 30S subunit to prevent premature association with the 50S subunit.

2005

**120.** Arrange the following steps of eukaryotic translation elongation in the correct order.
I. $eEF_1A$-GTP delivers aminoacyl-tRNA to the A-site.
II. Peptidyl transferase catalyzes peptide bond formation.
III. eEF2-GTP hydrolyzes GTP to promote translocation.
IV. Uncharged tRNA exits from the E-site.
(A) I, II, III, IV
(B) II, I, III, IV
(C) I, III, II, IV
(D) IV, I, II, III

2025

**121.** What is the most appropriate description of $eRF_1$ function?
(A) It is a GTPase that recycles the eukaryotic ribosome.
(B) It recognizes all three stop codons in eukaryotes and triggers peptide release.
(C) It binds specifically to the A-site to prevent premature termination.
(D) It structurally mimics the Kozak sequence.

2025

**122.** Which is a characteristic of toxins like Ricin targeting protein synthesis?
(A) They completely degrade mRNA molecules.
(B) They depurinate a specific universally conserved adenine in the 28S rRNA, halting elongation.
(C) They act as competitive inhibitors for aminoacyl-tRNA synthetases.
(D) They destroy the 5' cap.

2011

**123.** Identify the INCORRECT statement regarding the GroEL-GroES chaperone system.
(A) It primarily folds proteins before they are translated by the ribosome.
(B) GroEL forms two stacked heptameric rings.
(C) GroES acts as a "lid" that caps the GroEL cylinder.
(D) The complex requires ATP binding and hydrolysis for its conformational cycle.

2004

**124.** Which post-translational modification is explicitly associated with controlling the cell cycle and signal transduction cascades?
(A) N-linked glycosylation
(B) Irreversible proteolytic cleavage of signal peptides
(C) Reversible protein phosphorylation
(D) C-terminal amidation

2008

**125.** Which sequence accurately describes the steps of the secretory pathway?
(A) Rough ER → COPII vesicles → Golgi → Secretory vesicles
(B) Golgi → Rough ER → Lysosomes → Plasma Membrane
(C) Secretory vesicles → Golgi → Smooth ER → Nucleus
(D) Rough ER → Mitochondria → Golgi → Cell exterior

2024

**126.** What inference is valid regarding a mutant lac operon lacking a functional lac repressor (LacI-)?
(A) The operon will be permanently silenced.
(B) RNA polymerase will be permanently blocked.
(C) The operon will be constitutively expressed, provided glucose is absent (and cAMP is high).
(D) B-galactosidase will digest the repressor gene.

NODIA

2006

**127.** Which cause and effect relationship correctly characterizes Trp operon attenuation?
(A) High tryptophan → Ribosome stalls at Trp codons → Terminator forms → Transcription halts
(B) Low tryptophan → Ribosome stalls at Trp codons → Antiterminator forms → Transcription continues
(C) Low tryptophan → Repressor binds → Antiterminator forms → Translation accelerates
(D) High tryptophan → Ribosome speeds up → Antiterminator forms → Transcription

continues

2015

**128.** Identify the correct pair of a eukaryotic regulatory element and its function.
(A) Enhancer - Destabilizes the mRNA transcript
(B) Insulator - Connects the spliceosome to the transcription complex
(C) TATA box - Synthesizes the poly-A tail
(D) Core promoter - Determines the exact initiation site of transcription

2015

**129.** Which factual point defines ATP-dependent chromatin remodeling complexes?
(A) They utilize ATP hydrolysis to slide, eject, or restructure nucleosomes, altering DNA accessibility.
(B) They covalently modify histone tails by adding methyl groups.
(C) They are exclusively involved in maintaining telomere length.
(D) They act as primary components of the spliceosome.

2016

**130.** Which is the LEAST RELEVANT function of the 3' poly-A tail?
(A) Facilitating mRNA export from the nucleus
(B) Enhancing translation efficiency
(C) Protecting the mRNA from 3'-to-5' exonucleases
(D) Dictating the correct reading frame for the ribosome

2019

**131.** What defines the lariat structure formed during pre-mRNA splicing?
(A) A branched RNA molecule featuring a 2'-5' phosphodiester bond at the branch point adenosine
(B) A circular DNA intermediate
(C) A strictly linear piece of excised non-coding RNA
(D) A complex of multiple snRNPs bound to the promoter

2008

**132.** Which classification applies to RNA editing by cytidine deaminase in plants?
(A) Epigenetic DNA modification
(B) Transcriptional gene silencing
(C) Alternative splicing of pre-mRNA
(D) Post-transcriptional alteration of the mRNA coding sequence

2006

**133.** Identify the INCORRECT statement regarding the RISC complex.
(A) It incorporates a single-stranded guide RNA.
(B) It primarily functions in the nucleus to inhibit transcription.
(C) The Argonaute protein provides the catalytic cleavage "slicer" activity.
(D) It can induce either mRNA degradation or translational repression.

2024

**134.** Which advantage makes metagenomics specifically powerful for plant bioprospecting?
(A) It restricts analysis solely to heavily cultivated agricultural soils.
(B) It relies entirely on morphological characteristics of plant species.
(C) It allows access to the genetic material of unculturable microbes for novel gene discovery.
(D) It is fundamentally cheaper than conventional selective breeding.

2019

**135.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Transgenic metabolic engineering can effectively enrich staple crops with essential micronutrients.
Reason (R): Introduction of multiple genes can successfully reconstitute entire biochemical pathways, such as provitamin A synthesis, in target tissues.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**136.** What is the most appropriate methodological requirement for effective gene pyramiding?
(A) Robust molecular markers linked to each target gene must be available for accurate selection.
(B) The genes must be highly homologous and tandemly arrayed.
(C) The target genes must provide resistance to entirely unrelated abiotic stresses only.
(D) The pyramided crop must be sterile to prevent gene flow.

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2018

**137.** Which concept application accurately reflects the purpose of the GST-tag fusion system?
(A) It causes the protein to heavily precipitate, aiding in centrifugation.
(B) It allows single-step affinity purification of the fusion protein using a glutathione-sepharose column.
(C) It inherently refolds misfolded proteins without chaperones.
(D) It serves strictly as a fluorescent marker for confocal microscopy.

2003

**138.** Identify the defining feature of ribozymes.
(A) They are proteins that degrade RNA.
(B) They possess intrinsic catalytic activity strictly dependent on their RNA tertiary structure.
(C) They are purely structural elements of the cytoskeleton.
(D) They require exclusively lipid cofactors for activity.

2024

**139.** Which mechanism explains how tRNA aminoacylation proofreading maintains translational fidelity?
(A) It hydrolyzes the ester bond of mispaired aminoacyl-tRNAs before they enter the ribosome.
(B) It physically ejects incorrect amino acids from the A-site of the ribosome.
(C) It degrades mRNAs containing rare codons.
(D) It chemically alters the anticodon to match the attached amino acid.

2021

**140.** Arrange the following events of eukaryotic translation initiation in the proper sequence.
I. Binding of initiator tRNA to the 40S subunit.
II. Recognition of the 5' cap by the eIF4F complex.
III. Scanning of the mRNA for the Kozak sequence.
IV. Joining of the 60S large ribosomal subunit.
(A) II, I, III, IV
(B) I, II, III, IV
(C) II, III, I, IV
(D) I, III, II, IV

2008

**141.** Identify the factual distinction between Class I and Class II aminoacyl-tRNA synthetases.
(A) Class I utilizes an RNA core; Class II uses a protein core.
(B) Class I enzymes are strictly eukaryotic; Class II are prokaryotic.
(C) Class I recognizes stop codons; Class II recognizes start codons.
(D) Class I attaches the amino acid to the 2' OH; Class II to the 3' OH of the terminal adenosine.

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2005

**142.** Which point accurately characterizes the primary function of the Shine-Dalgarno sequence?
(A) It dictates the site of intrinsic transcription termination.
(B) It recruits the eukaryotic 40S subunit.
(C) It base-pairs with the 16S rRNA to align the start codon in the P-site.
(D) It acts as the binding site for the Lac repressor.

2021

**143.** Given below are certain statements regarding elongation factors.
I. EF-Tu binds directly to the E-site.
II. $eEF_1A$ is the eukaryotic homolog of EF-Tu.
III. EF-Ts acts as a guanine nucleotide exchange factor (GEF) for EF-Tu.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I

2010

**144.** What defines the specific catalytic action of 23S rRNA in the ribosome?
(A) It acts as an endonuclease to cleave incorrectly matched mRNA-tRNA pairs.
(B) It cleaves GTP to provide the necessary energy for ribosomal translocation.
(C) It actively synthesizes ATP during the elongation phase of protein synthesis.
(D) It aligns tRNAs to facilitate peptidyl transfer via an induced-fit mechanism.

2013

**145.** Which is the LEAST RELEVANT element for eukaryotic translation termination?
(A) eIF4G
(B) eRF3
(C) A stop codon in the A-site
(D) $eRF_1$

2015

**146.** Which cause and effect relationship defines the toxicity of a specific antibiotic?
(A) Chloramphenicol binds the 40S subunit

(C) The ribosome slides bidirectionally until it locates an internal ribosome entry site (IRES).
(D) The scanning process is powered exclusively by ATP hydrolysis from the 18S rRNA.

2023

**164.** What inference is valid regarding the function of $eEF_1B$ in eukaryotic elongation?
(A) It actively cleaves the peptide bond to correct errors.
(B) It physically forces the uncharged tRNA out of the E-site.
(C) It functions as a specialized release factor for rare codons.
(D) It acts as a GEF, regenerating the active $eEF_1A$-GTP complex from $eEF_1A$-GDP.

2010

**165.** Match List I with List II.
List I: (Molecular Components)
P. Dicer
Q. TATA-binding protein
R. eEF2
S. $eRF_1$
List II: (Role)
1. Eukaryotic translocation
2. Recognition of eukaryotic stop codons
3. Processing of double-stranded RNA
4. Basal transcription initiation
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-1, Q-2, R-3, S-4
(D) P-3, Q-4, R-2, S-1

2018

**166.** Which is an EXCEPTION among translation inhibitors regarding their target domain?
(A) Chloramphenicol targets the 50S subunit.
(B) Erythromycin targets the 50S subunit.
(C) Diphtheria toxin targets the elongation factor eEF2.
(D) Tetracycline targets the 30S subunit.

2025

**167.** Identify the factual characteristic of protein prenylation.
(A) It heavily targets nuclear structural proteins exclusively.
(B) It targets an N-terminal glycine.
(C) It occurs entirely independent of cellular lipid synthesis pathways.
(D) It involves the covalent attachment of farnesyl or geranylgeranyl isoprenoid groups to a C-terminal cysteine.

2007

**168.** What is the most appropriate description of the signal recognition particle (SRP)?
(A) A ribonucleoprotein complex that recognizes signal sequences and targets ribosomes to the rough ER
(B) A strictly proteinaceous kinase that phosphorylates newly synthesized peptides
(C) A cytoplasmic protease that specifically destroys signal sequences
(D) A specialized tRNA that transports hydrophobic amino acids

2004

**169.** Which cause and effect relationship describes catabolite repression in the lac operon?
(A) High glucose → High cAMP → CAP binds promoter → Transcription increases
(B) High glucose → Low cAMP → CAP does not bind promoter → Transcription is severely reduced
(C) Low glucose → Low cAMP → CAP cannot bind → Transcription stops
(D) Low glucose → High cAMP → Repressor permanently detaches → Transcription maximizes regardless of lactose

2025

**170.** Which sequence of structural events correctly initiates the Trp operon attenuation termination?
(A) Ribosome stalls → Region 1 pairs with 2 → Region 3 pairs with 4
(B) Ribosome translates quickly → Region 2 pairs with 3 → Region 4 remains single-stranded
(C) Ribosome translates quickly → Region 2 is blocked → Region 3 pairs with 4 → Terminator forms
(D) Ribosome stalls → Region 3 pairs with 4 → RNA polymerase detaches

2012

**171.** Identify the INCORRECT statement regarding eukaryotic enhancers.
(A) Their functional capability strictly relies on their orientation relative to the promoter.
(B) They are composed of multiple binding sites for various transcription factors.
(C) They can exert their influence over extremely large genomic distances (megabases).
(D) They often act through DNA looping mediated by the Mediator complex.

2022

**172.** What is the primary function of the Mediator complex in eukaryotic transcription?

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Assertion (A): RNA interference via siRNAs is considered a form of post-transcriptional gene silencing (PTGS).
Reason (R): siRNAs guide the cleavage of mature, already-transcribed target mRNAs in the cytoplasm.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**156.** Which is an advantage of targeted RNA editing via artificial A-to-I deaminases?
(A) It permanently alters the host's genomic DNA.
(B) It entirely avoids the use of guide RNAs.
(C) It can correct disease-causing point mutations at the transcript level without permanent genomic modification.
(D) It eliminates the need for any cellular translation machinery.

2023

**157.** What defines the action of a hammerhead ribozyme?
(A) It splices out large introns from ribosomal RNA.
(B) It requires multiple protein subunits to achieve a catalytically active conformation.
(C) It strictly synthesizes small interfering RNAs.
(D) It catalyzes site-specific cleavage of its target RNA via a transesterification reaction.

2021

**158.** Which concept application best describes the role of multi-gene vectors in plant biotechnology?
(A) They ensure the destruction of endogenous plant defenses.
(B) They specifically induce random mutagenesis across the plant genome.
(C) They allow for the simultaneous integration of an entire targeted metabolic pathway (e.g., biofortification) in a single transformation event.
(D) They replace the need for traditional selectable markers entirely.

2025

**159.** Which of the following statements about histidine tags (His-tags) is INCORRECT?
(A) They typically consist of a sequence of six consecutive histidine residues.
(B) They require the presence of strong detergents to bind to the affinity column.
(C) They coordinate tightly with immobilized divalent metal ions like $Ni^{2+}$ or $Co^{2+}$.
(D) The bound protein is usually eluted using imidazole.

2010

**160.** Given below are certain statements about a chimeric reporter construct.
I. A functional promoter sequence
II. An uninterrupted open reading frame of the reporter gene
III. A highly robust origin of replication for eukaryotic cells
IV. A transcriptional terminator sequence
Which of the above elements are necessary for proper functioning?
(A) I, III and IV
(B) II, III and IV
(C) I, II and III
(D) I, II and IV

2007

**161.** What defines the structural difference between the tRNA cloverleaf and its L-shape?
(A) The cloverleaf represents 2D secondary structure, while the L-shape represents the 3D tertiary structure.
(B) The cloverleaf is a primary sequence, while the L-shape is a purely random coil.
(C) The cloverleaf exists only in the nucleus; the L-shape exists only in the mitochondria.
(D) The cloverleaf is fully devoid of modified bases compared to the L-shape.

2023

**162.** Identify the correct pair of molecular factors regulating prokaryotic translation initiation.
(A) EF-G - Binds to the Shine-Dalgarno sequence
(B) Release Factor 1 - Assembles the 50S subunit
(C) IF-1 - Enhances IF-2 binding and stabilizes the 30S complex
(D) IF-3 - Delivers the charged tRNA to the A-site

2017

**163.** Which mechanism details the ribosomal scanning process in eukaryotes?
(A) The 43S pre-initiation complex moves 5'-to-3' from the cap until it encounters the AUG start codon within a Kozak consensus.
(B) The 60S subunit moves 3'-to-5' seeking a stop codon.

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2021

**196.** Which is a characteristic defining the difference between Group I and Group II self-splicing introns?

(A) Group I is strictly found in animals; Group II is exclusively in plants.
(B) Group I relies entirely on a large ribonucleoprotein complex; Group II is purely autocatalytic.
(C) Group I occurs only in transfer RNAs; Group II only in messenger RNAs.
(D) Group I utilizes an external guanosine nucleophile; Group II uses an internal adenosine to form a lariat.

2012

**197.** What defines the specific function of poly(A) polymerase?

(A) It copies a poly-T template from the DNA genome to add the poly-A tail.
(B) It adds adenine nucleotides to the 3' end of the mRNA strictly without utilizing a nucleic acid template.
(C) It synthesizes the poly-A tail directly during the elongation phase of transcription.
(D) It degrades the mRNA from the 3' end.

2017

**198.** Which inference about a protein lacking any post-translational modifications is most accurate?

(A) It will undoubtedly fold faster than a heavily modified protein.
(B) It is definitively an extracellular matrix component.
(C) It may lack the complex regulatory switches or specific subcellular targeting signals provided by modifications.
(D) It will permanently reside strictly within the nucleolus.

2002

**199.** Identify the primary role of the ubiquitin chain.

(A) To direct the tagged protein to the lysosome
(B) To assist the protein in translocating across the ER membrane
(C) To serve as an alternative energy source to ATP
(D) To act as a specific recognition signal for the 26S proteasome to degrade the tagged protein

2012

**200.** Given below are certain statements concerning translation initiation factors and their functions.

I. eIF4E specifically binds the 5' m7G cap.
II. eIF4A functions as an ATP-dependent RNA helicase to unwind secondary structures.
III. eIF2 binds the poly-A tail.
IV. IF-2 (prokaryotic) utilizes GTP.

Which of the above statement(s) is/are correct?

(A) I, II and IV
(B) II, III and IV
(C) I, III and IV
(D) I and II

2001

**201.** Identify the factual distinction regarding codon usage.

(A) Synonymous codons usually differ only in their first nucleotide.
(B) Codon usage bias strongly correlates with the abundance of corresponding isoaccepting tRNAs in highly expressed genes.
(C) Rare codons typically accelerate the rate of translation elongation.
(D) Stop codons are completely immune to any context-dependent bias.

2021

**202.** What is the most appropriate characterization of the TΨC loop in tRNA?

(A) It actively forms the peptide bond.
(B) It interacts with the large ribosomal subunit to facilitate proper tRNA docking.
(C) It directly base-pairs with the mRNA codon.
(D) It serves as the covalent attachment point for the amino acid.

NODIA

2016

**203.** Which mechanism details how EF-G promotes translocation?

(A) It binds to the A-site post-peptidyl transfer, and its GTP hydrolysis drives a major conformational change that shifts the tRNAs into the P and E sites.
(B) It cleaves the mRNA, pulling it through the ribosome.
(C) It heavily methylates the newly formed peptide bond.
(D) It simply blocks the entry of the next aminoacyl-tRNA until translocation spontaneously occurs.

2014

**204.** Which concept application describes the utility of studying translational inhibitors like puromycin?

(A) They act as vital nutritional supplements for bacterial cultures.
(B) They inherently reverse the direction of translation to correct errors.
(C) They serve as indispensable structural probes

(A) It acts as a molecular bridge between distal enhancers and the core promoter complex.
(B) It functions as the primary RNA polymerase for all mitochondrial genes.
(C) It facilitates the degradation of improperly spliced pre-mRNA transcripts.
(D) It adds the 3' poly-A tail to the mature mRNA transcript.

2014

**173.** What defines the specific role of snRNPs in the spliceosome?
(A) They act solely as structural scaffolds holding the DNA open.
(B) They synthesize the poly-A tail during splicing.
(C) They strictly transport the mature mRNA out of the nucleus.
(D) They recognize splice sites via RNA-RNA base pairing and facilitate the catalytic splicing reactions.

2023

**174.** Which concept application justifies the engineering of specific alternative splicing pathways in crops?
(A) To entirely eliminate the crop's natural immune system
(B) To permanently fuse the entire genome into a single chromosome
(C) To generate distinct protein isoforms that provide variable resistance traits under different environmental stresses
(D) To bypass the requirement for ribosomal translation

2024

**175.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Metagenomic bioprospecting requires robust high-throughput screening technologies.
Reason (R): The vast majority of cloned DNA fragments from environmental samples will not encode the specific desired functional activity.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**176.** Given below are certain statements regarding Golden Rice biofortification.
I. It utilizes a phytoene synthase gene derived from daffodil or maize.
II. It requires a carotene desaturase gene typically sourced from bacteria (Erwinia).
III. It naturally accumulates massive amounts of vitamin C alongside provitamin A.
IV. The target tissue for accumulation is the starchy endosperm.
Which of the above statement(s) is/are correct?
(A) II, III and IV
(B) I, III and IV
(C) I and II
(D) I, II and IV

2007

**177.** Which is a recognized advantage of marker-assisted selection (MAS) in gene pyramiding?
(A) It enables the identification of plants carrying multiple resistance genes even when their individual phenotypes are indistinguishable.
(B) It fundamentally increases the natural mutation rate of the crop.
(C) It ensures absolute immunity against all conceivable future pathogens.
(D) It completely bypasses the traditional requirement for crossing plants.

2014

**178.** Identify the correct pair describing a reporter gene and its specific utility.
(A) Luciferase - Used primarily for generating antibiotic-resistant clones
(B) GFP - Requires complex external cofactors and destructive cell lysis to visualize
(C) GUS (β-glucuronidase) - Commonly used for histochemical localization in plant tissues
(D) Chloramphenicol acetyltransferase (CAT) - Produces a bright fluorescent signal in live cells

2021

**179.** What inference is valid regarding the wobble hypothesis?
(A) Mutations in the third codon position are less likely to result in an amino acid change (synonymous mutations).
(B) The ribosome structurally wobbles during translocation, occasionally skipping codons.
(C) The first codon position is entirely degenerate and irrelevant.
(D) It strictly applies only to eukaryotic organisms.

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→ Blocks the scanning process → Halts initiation
(B) Puromycin binds the E-site → Prevents uncharged tRNA exit → Ribosome stalls
(C) Tetracycline binds the 30S subunit → Prevents aminoacyl-tRNA from entering the A-site → Halts elongation
(D) Cycloheximide binds the 30S subunit → Causes severe misreading → Produces truncated proteins

2004

**147.** Identify the correct pair linking the post-translational modification to its primary cellular consequence.
(A) Ubiquitination (poly) - Targeted protein degradation
(B) Methylation - Immediate secretion from the cell
(C) Glycosylation - Targeted to the nucleus
(D) Acetylation - Attachment to the plasma membrane

2013

**148.** Which EXCEPTION exists regarding the signal peptide hypothesis?
(A) Most secretory proteins have their signal sequence cleaved co-translationally.
(B) Signal peptides are predominantly hydrophobic.
(C) The SRP pauses translation until the ribosome docks at the ER membrane.
(D) All nuclear-encoded mitochondrial proteins utilize the exact same ER-targeting signal peptide.

2025

**149.** What inference can be drawn from the presence of a zinc finger domain in a newly discovered protein?
(A) It is primarily an extracellular structural protein.
(B) It is likely a nucleic-acid binding protein or transcription factor.
(C) It function as an ATP-dependent membrane transporter.
(D) It is an enzyme responsible for degrading lipids.

2024

**150.** Which mechanism explicitly controls the negative regulation of the lac operon?
(A) The binding of cAMP to CAP
(B) The rapid degradation of the lac mRNA
(C) The physical binding of the LacI repressor to the operator sequence
(D) The heavy methylation of the promoter region

2013

**151.** Identify the core feature of the Trp operon attenuator.
(A) It relies on a specific ribonuclease to cleave the transcript.
(B) It strictly requires the cAMP-CAP complex to function.
(C) It is regulated by the binding of a small RNA (sRNA).
(D) It involves the formation of alternative RNA secondary structures depending on translation speed.

2018

**152.** Which feature characterizes a typical leucine zipper domain?
(A) Two $\alpha$ helices forming a coiled-coil dimer via periodic leucine residues
(B) A $\beta$-barrel with hydrophobic interior
(C) A single $\alpha$ helix coordinated by a structural zinc ion
(D) A highly disordered loop binding to RNA

2005

**153.** What is the most appropriate definition of the "histone code" hypothesis?
(A) Histones code for specific transfer RNAs.
(B) Specific combinations of post-translational histone modifications dictate chromatin structure and transcriptional states.
(C) Histone proteins are the sole determining factor in alternative splicing.
(D) The exact sequence of histone variants directly replaces the DNA genetic code.

2003

**154.** Identify the factual outcome of alternative splicing.
(A) It inherently causes genomic instability and double-strand breaks.
(B) It generates multiple distinct mature mRNAs and protein isoforms from a single primary transcript.
(C) It fully bypasses the requirement for RNA polymerase II.
(D) It completely halts all translation in the cytoplasm.

2001

**155.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).

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residues for dimerization.
(C) It consists of two α helices, one of which specifically fits into the major groove of DNA.
(D) It functions solely in the cytoplasm to tether proteins to the membrane.

2005

**189.** Which mechanism directly links DNA methylation to transcriptional silencing?
(A) Methyl groups heavily attract RNA polymerase.
(B) Methylation strictly causes immediate degradation of the core promoter sequence.
(C) Methyl groups physically block the assembly of the spliceosome.
(D) Methylated cytosines serve as binding sites for methyl-CpG-binding domain (MBD) proteins, which recruit histone deacetylases.

2009

**190.** Given below are certain statements about RNA editing.
I. C-to-U editing is frequently observed in plant mitochondria and chloroplasts.
II. A-to-I editing is catalyzed by the ADAR enzyme family.
III. RNA editing always occurs exactly at the transcription start site.
Which of the above statement(s) is/are correct?
(A) II and III
(B) I and III
(C) I and II
(D) I

2011

**191.** Identify the factual outcome of a cell lacking functional Dicer enzyme.
(A) Primary transcripts (pri-miRNAs) cannot be synthesized.
(B) The generation of manual siRNAs and miRNAs from their double-stranded precursors will be severely impaired.
(C) RNA polymerase II will be permanently inhibited.
(D) The ribosome will fail to assemble the 40S and 60S subunits.

2014

**192.** Arrange the following steps to isolate a novel bioactive gene via metagenomics in the correct order.
I. Environmental sample extraction
II. Metagenomic DNA isolation
III. Library construction
IV. Functional screening
V. Gene isolation
(A) I, II, III, IV, V
(B) III, I, IV, II, V
(C) V, II, I, III, IV
(D) IV, III, V, I, II

2002

**193.** Which cause and effect relationship defines the success of ferritin overexpression in biofortification?
(A) Ferritin overexpression → Increased chelation and safe storage of iron in plant tissues → Higher bioavailable iron content
(B) Ferritin overexpression → Total blockade of iron uptake from soil → Severe chlorosis
(C) Ferritin overexpression → Accumulation of β-carotene → Golden coloration
(D) Ferritin overexpression → Accelerated degradation of zinc → Zinc deficiency

2013

**194.** Identify the INCORRECT statement regarding the application of ribozymes in biotechnology.
(A) They can be engineered to specifically target and downregulate pathological mRNAs.
(B) Motif designs like the hammerhead can be incorporated into customized antisense constructs.
(C) They offer an alternative approach to RNA interference for gene silencing.
(D) They are fundamentally limited because they act strictly as non-recyclable suicidal molecules.

2021

**195.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Green Fluorescent Protein (GFP) requires no exogenous cofactors for its fluorescence.
Reason (R): Its fluorophore is formed autocatalytically by the spontaneous cyclization and oxidation of three specific internal amino acids.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2012

**180.** Which mechanism ensures that each tRNA is attached to its correct amino acid?
(A) The ribosome physically measures the size of each amino acid.
(B) Spontaneous chemical affinity between the amino acid and the anticodon.
(C) Pre-folding of the tRNA around the specific amino acid in the nucleus.
(D) Specific recognition of the tRNA's anticodon loop and acceptor stem by its cognate aminoacyl-tRNA synthetase.

2013

**181.** What is the function of the E-site in the ribosome?
(A) It is the entry site for all incoming aminoacyl-tRNAs.
(B) It is the exit site where uncharged, deacylated tRNA temporarily binds before leaving the ribosome.
(C) It holds the growing polypeptide chain.
(D) It strictly binds the release factors during termination.

2001

**182.** Which structural feature explicitly differentiates the eukaryotic 5' mRNA cap from a standard nucleotide?
(A) It consists of an unmethylated adenine base.
(B) It contains a strictly 3'-to-5' phosphodiester linkage.
(C) It is linked to the first mRNA nucleotide via an unusual 5'-to-5' triphosphate bridge.
(D) It lacks a sugar moiety completely.

2009

**183.** Identify the INCORRECT statement regarding translation termination.
(A) Release factors structurally mimic the shape of a tRNA molecule to enter the A-site.
(B) Hydrolysis of the ester bond linking the polypeptide to the tRNA is catalyzed by the peptidyl transferase center.
(C) Eukaryotes utilize a single release factor ($eRF_1$) to recognize all three stop codons.
(D) Prokaryotes do not require GTP hydrolysis for the action of RF3.

2014

**184.** What defines the primary role of molecular chaperones like Hsp70?
(A) They bind transiently to exposed hydrophobic patches on non-native proteins to prevent aggregation.
(B) They specifically target natively folded proteins for immediate degradation.
(C) They actively unfold correctly folded proteins to regulate their activity.
(D) They serve exclusively as structural components of the nuclear pore.

2016

**185.** Match List I with List II.
List I: (Post-translational Modification)
P. N-linked Glycosylation
Q. Phosphorylation
R. Acetylation
S. O-linked Glycosylation
List II: (Target Amino Acid)
1. Lysine
2. Serine/Threonine (hydroxyl group)
3. Asparagine
4. Serine/Threonine/Tyrosine
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-3, Q-2, R-1, S-4
(D) P-1, Q-4, R-3, S-2

2007

**186.** Which concept application describes the utility of signal peptide cleavage during recombinant protein production?
(A) It traps the recombinant protein within the inclusion bodies.
(B) It allows the host cell to secrete the mature, processed recombinant protein into the culture medium, simplifying purification.
(C) It guarantees the immediate ubiquitination of the protein.
(D) It actively represses transcription of the recombinant gene.

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2008

**187.** Which is the LEAST RELEVANT regulatory mechanism for controlling the precise initiation frequency of the lac operon?
(A) The rate of intrinsic transcription termination at the end of the lacA gene
(B) The intracellular concentration of cAMP
(C) The binding affinity of the Lac repressor to the operator
(D) The exact sequence conservation of the core promoter relative to the consensus

2008

**188.** What is the most appropriate description of a helix-turn-helix domain?
(A) It coordinates a zinc ion to bind exclusively to single-stranded RNA.
(B) It relies on heavily methylated leucine

to dissect the distinct steps and functional centers of the ribosome.
(D) They are exclusively used to enhance the speed of in vitro translation.

2007

**205.** Match List I with List II.
List I: (Genetic Regulatory Element)
P. Cis-acting element
Q. Trans-acting factor
R. Operon
S. Epigenetic mark
List II: (Definition)
1. A cluster of co-regulated genes transcribed as a single polycistronic mRNA
2. A diffusible protein that binds to specific DNA sequences
3. A reversible modification on DNA or histones affecting gene expression
4. A DNA sequence that regulates the expression of genes located on the same continuous DNA molecule
(A) P-2, Q-4, R-3, S-1
(B) P-1, Q-3, R-2, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-4, Q-2, R-1, S-3

2011

**206.** Which is an EXCEPTION to the strict definition of an enhancer?
(A) It functions effectively regardless of its orientation.
(B) It can be located thousands of base pairs away from the transcription start site.
(C) It strictly initiates transcription entirely independent of a core promoter.
(D) It contains specific binding motifs for transcriptional activator proteins.

2018

**207.** Identify the INCORRECT pair regarding epigenetic modifications.
(A) Histone Acetyltransferase (HAT) - Generally associated with transcriptional activation
(B) DNA Methyltransferase (DNMT) - Generally associated with transcriptional activation
(C) Histone Deacetylase (HDAC) - Generally associated with transcriptional repression
(D) ATP-dependent remodeling complex - Modifies nucleosome spacing

2024

**208.** What inference can be made about a pre-mRNA lacking a functional branch point sequence?
(A) The mature mRNA will be translated significantly faster.
(B) The snRNPs will skip the intron and splice the adjacent exons with higher efficiency.
(C) The transcript will undergo self-splicing instead.
(D) The first transesterification step of splicing, forming the lariat, cannot occur.

2025

**209.** Which cause and effect relationship correctly outlines alternative splicing?
(A) Differential inclusion of exons → Variable mRNA sequences → Production of diverse protein isoforms from one gene
(B) Random deletion of introns → Complete loss of genomic DNA → Cell death
(C) Alternative promoter usage → Altered reading frame → Premature termination exclusively
(D) Heavy methylation of exons → Prevention of translation → Severe reduction in proteome diversity

2015

**210.** Given below are certain statements about siRNAs.
I. They are derived from long double-stranded RNA precursors.
II. They typically guide perfect complementary cleavage of target mRNA.
III. They are structurally identical to transfer RNAs.
Which of the above statement(s) is/are correct?
(A) II and III
(B) I and III
(C) I and II
(D) I

2013

**211.** Identify the factual benefit of isolating promoter sequences from wild plant relatives.
(A) They may offer specific stress-inducible regulatory patterns adapted to extreme environmental conditions.
(B) They always drive constitutive, extremely high-level expression across all tissue types.
(C) They are completely immune to any form of epigenetic silencing.
(D) They inherently synthesize novel proteins without a coding sequence.

2022

**212.** What defines the specific genetic intervention required for increasing bioavailable iron via the nicotianamine synthase pathway?

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2017

**227.** What defines the specific limitation of using ribozymes as direct therapeutic agents?
(A) They are highly susceptible to degradation by ubiquitous cellular and extracellular ribonucleases.
(B) They are inherently highly stable and persist forever in the bloodstream.
(C) They spontaneously convert into infectious viral particles.
(D) They permanently alter the genetic code of the host cell.

2001

**228.** Which recall-based statement concerning translation is correct?
(A) The peptidyl transferase center is located in the small ribosomal subunit.
(B) The initiator tRNA in eukaryotes carries a formylated methionine.
(C) Stop codons are typically recognized by specialized stop-tRNAs.
(D) The start codon sets the exact reading frame for the entire subsequent translation process.

2001

**229.** Identify the correct pair describing a component of the secretory pathway.
(A) Sec61 complex - Mitochondrial membrane pore for protein entry
(B) SRP - Cytoplasmic ribonucleoprotein that recognizes the signal peptide
(C) Signal peptidase - Cytoplasmic enzyme that destroys mature proteins
(D) COPII vesicles - Transport proteins specifically from the Golgi to the ER

2003

**230.** Given below are certain statements regarding epigenetic modifications.
I. They are entirely dependent on altering the primary DNA sequence.
II. They are potentially heritable through cell divisions.
III. They play a critical role in cellular differentiation.
IV. They are completely impervious to environmental influences.
Which of the above statement(s) is/are correct?
(A) II and III
(B) I and II
(C) III and IV
(D) I, II and IV

2019

**231.** What is the function of the 3' UTR in mature eukaryotic mRNA?
(A) It codes for the final few amino acids of the protein.
(B) It functions exclusively to inhibit polyadenylation.
(C) It inherently splices itself out before translation begins.
(D) It often contains regulatory sequences that influence mRNA stability, localization, and translational efficiency.

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2007

**232.** What is the most appropriate definition of gene pyramiding in agricultural biotechnology?
(A) The systematic assembly of multiple desirable genes, typically for resistance or quality traits, into a single elite genotype
(B) The process of randomly scattering seeds from different varieties in a single field
(C) The construction of highly complex, pyramid-shaped greenhouse environments
(D) The gradual elimination of wild-type alleles through continuous inbreeding

2007

**233.** Which concept application justifies the inclusion of a proteolytic cleavage site between an affinity tag and the target protein?
(A) To guarantee the permanent stability of the fusion protein
(B) To allow for the gentle, site-specific removal of the tag after purification, yielding native target protein
(C) To explicitly prevent the protein from folding correctly
(D) To serve as an alternative translation initiation site

2003

**234.** Which mechanism correctly details how miRNAs usually cause translational repression?
(A) They completely block the transcription of the target gene.
(B) They guide the RISC complex to bind imperfectly to the 3' UTR of target mRNAs, heavily impeding ribosomal progression or initiation.
(C) They actively transport the target mRNA directly into the lysosome.
(D) They specifically degrade the 5' cap structure in the nucleus.

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Which of the above statement(s) is/are correct?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I and II

2016

**220.** Arrange the following key events of the Trp operon attenuation mechanism in chronologically correct order for a situation where tryptophan is abundant.
I. RNA polymerase begins transcribing the leader sequence.
II. Ribosome quickly translates the adjacent Trp codons in region 1.
III. Region 3 base-pairs with region 4 to form the terminator hairpin.
IV. RNA polymerase dissociates before reaching the structural genes.
(A) I, II, III, IV
(B) I, III, II, IV
(C) II, I, III, IV
(D) I, II, IV, III

2019

**221.** Which factual constraint strictly applies to DNA methylation in eukaryotic gene regulation?
(A) It exclusively targets the adenine residue in the TATA box.
(B) It fundamentally relies on the constant presence of the ribosome in the nucleus.
(C) It completely prevents the DNA from being replicated during S phase.
(D) It typically occurs on the cytosine residue within a CpG dinucleotide context.

2010

**222.** What inference is valid regarding the evolutionary origin of Group I and II introns?
(A) They clearly evolved strictly from modern viral reverse transcriptases.
(B) They are purely synthetic artifacts of modern genetic engineering.
(C) Because they possess intrinsic catalytic activity (ribozymes), they are considered remnants of the ancestral "RNA World."
(D) They evolved primarily to synthesize complex lipid bilayers.

2010

**223.** Identify the INCORRECT statement regarding the RNA-Induced Silencing Complex (RISC).
(A) It utilizes the guide strand to locate complementary mRNA targets.
(B) It acts as a powerful transcriptional enhancer when bound to mRNA.
(C) The passenger strand of the siRNA duplex is typically discarded during RISC activation.
(D) It contains a member of the Argonaute protein family as its catalytic core.

2005

**224.** Which is the most appropriate target for metagenomic bioprospecting aimed at discovering novel cold-active industrial enzymes?
(A) Standard agricultural topsoil from tropical regions
(B) Soil samples strictly retrieved from actively erupting volcanoes
(C) Pure cultures of standard laboratory E. coli strains
(D) Environmental samples collected from polar ice caps or deep-ocean thermal vents

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2015

**225.** Match List I with List II.
List I: (Structural or Functional Trait)
P. Kozak sequence
Q. $eEF_1A$
R. Hsp60
S. Shine-Dalgarno sequence
List II: (Biological Molecule)
1. Found exclusively in prokaryotic mRNA
2. Provides an isolated chamber for protein folding
3. Found exclusively in eukaryotic mRNA
4. Facilitates eukaryotic tRNA delivery
(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-2, S-3

2012

**226.** Which cause and effect relationship correctly explains the functionality of the His-tag in protein purification?
(A) The His-tag introduces massive negative charges → The protein strictly binds to cation-exchange resins
(B) The His-tag fundamentally alters protein folding → The protein violently precipitates out of solution
(C) The polyhistidine sequence strongly coordinates transition metal ions → The fusion protein specifically binds to IMAC columns
(D) The His-tag binds glutathione → The protein is captured on glutathione-sepharose beads

**9.** (B) P-4, Q-1, R-2, S-3
Chaperones play distinct roles in maintaining protein homeostasis. Hsp70 binds hydrophobic regions of nascent peptides to prevent aggregation. GroEL is a double-ring chaperonin that provides a folding chamber, while GroES serves as the chamber's lid. Ubiquitin is a small protein that covalently attaches to target proteins, marking them for degradation by the proteasome to remove misfolded or unnecessary molecules.

**10.** (B) I and II
N-linked glycosylation is a post-translational modification where a pre-assembled oligosaccharide is attached to the amide nitrogen of an asparagine residue. This process occurs in the endoplasmic reticulum and requires the specific consensus sequence Asn-X-Ser/Thr. It is a critical step for proper protein folding and trafficking. Assembly in the Golgi refers to O-linked glycosylation or the subsequent pruning of N-linked chains.

**11.** (A) GTP hydrolysis by both the SRP and the SRP receptor
The signal recognition particle (SRP) targets nascent proteins to the endoplasmic reticulum. Upon binding of the SRP-ribosome complex to the SRP receptor on the membrane, both the SRP and its receptor act as GTPases. The mutual hydrolysis of GTP triggers a conformational change that releases the SRP from the ribosome, allowing the polypeptide to enter the translocon channel.

**12.** (C) Negative inducible and positive inducible
The lac operon exhibits dual regulation mechanisms to ensure efficient nutrient utilization. It is negatively inducible because the lac repressor prevents transcription unless lactose is present. Simultaneously, it is positively inducible via the cAMP-CAP complex, which enhances RNA polymerase recruitment when glucose levels are low. These overlapping controls allow the bacteria to prioritize glucose while still being able to metabolize lactose.

**13.** (D) Coupling of transcription and translation dynamics
Attenuation in the trp operon is a regulatory mechanism unique to prokaryotes because it requires the simultaneous occurrence of transcription and translation. The rate of the moving ribosome on the leader sequence determines which RNA secondary structures form. These structures either allow the RNA polymerase to continue or force it to terminate early, providing a sensitive response to cellular tryptophan levels.

**14.** (C) SH2 domain
Transcription factors use specific structural motifs to recognize and bind DNA sequences. Zinc fingers, helix-turn-helix, and leucine zippers are well-characterized DNA-binding domains. In contrast, SH2 domains are primarily involved in protein-protein interactions by recognizing phosphorylated tyrosine residues. Therefore, the SH2 domain does not play a direct role in sequence-specific DNA binding required for the regulation of gene transcription.

**15.** (B) The gene is transcriptionally repressed.
DNA methylation at cytosine residues in CpG islands is a major epigenetic signal for gene silencing. This modification often recruits proteins that attract histone deacetylases, which remove acetyl groups from histone tails. The resulting loss of acetylation promotes chromatin condensation into a heterochromatic state. This tightly packed structure physically prevents the transcriptional machinery from accessing the promoter, leading to repression.

**16.** (D) Guanylyltransferase
The 5' capping of eukaryotic pre-mRNA is a vital post-transcriptional modification. The process begins with an RNA triphosphatase removing a phosphate group, followed by the action of guanylyltransferase. This enzyme adds a GMP molecule in an unusual 5'-to-5' triphosphate linkage. Subsequent methylation by methyltransferase completes the structure, which protects the transcript from degradation and facilitates translation initiation by the ribosome.

**17.** (A) It increases proteome diversity from a limited number of genes.
Alternative splicing allows a single gene to produce multiple distinct protein isoforms by selectively including or excluding different exons. This mechanism significantly expands the functional capacity of the proteome without increasing the total number of genes in the genome. It enables different tissues or developmental stages to tailor protein functions to specific physiological needs through regulated changes in mRNA processing.

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2019

**235.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The genetic biofortification of crops offers a more sustainable long-term solution to micronutrient deficiency than dietary supplementation.
Reason (R): Once the biofortified seeds are distributed, the crop can be propagated by farmers, naturally enriching the local food supply continuously.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**236.** Which factual statement best describes the fundamental process of RNA splicing?
(A) The irreversible translation of RNA into a protein sequence
(B) The rapid degradation of mature mRNA by specific nucleases
(C) The highly precise removal of non-coding introns and the precise ligation of coding exons
(D) The addition of random non-templated nucleotides to the 5' end

2001

**237.** What defines the structural layout of a standard operon in prokaryotes?
(A) A single promoter strictly governing the transcription of a single gene
(B) Multiple distinct promoters driving an array of functionally unrelated genes
(C) A single regulatory promoter controlling the transcription of multiple contiguous structural genes into one mRNA
(D) A series of promoters each controlling its own downstream structural gene individually.

2001

**238.** Identify the INCORRECT point concerning the regulation of eukaryotic transcription.
(A) It involves highly complex interactions between distal enhancers and core promoters.
(B) It relies primarily on the physical looping of chromatin.
(C) It utilizes identical repressor mechanisms to the prokaryotic lac operon.
(D) It heavily utilizes the Mediator complex as a transcriptional coactivator.

2018

**239.** Which of the following is the LEAST RELEVANT application of the GUS reporter system?
(A) Determining the exact tissue-specific expression pattern of a novel plant promoter
(B) Tracking the rapid, real-time intracellular movement of a protein within a live, growing root
(C) Assessing the efficiency of Agrobacterium-mediated transient transformation
(D) Analyzing the developmental stage at which a specific gene is activated

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2008

**240.** Given below are certain elements about a functional hammerhead ribozyme.
I. Three base-paired stems (Helices I, II, and III)
II. A structurally conserved catalytic core of non-helical nucleotides
III. A mandatory requirement for a large protein co-enzyme
IV. A strict requirement for a divalent metal ion (often $Mg^{2+}$) for folding and maximal catalytic efficiency
V. The generation of a highly robust poly-A tail post-cleavage
Which of the above statement(s) is/are correct?
(A) II, III and V
(B) I, II, III and IV
(C) I, IV and V
(D) I, II and IV

2003

**241.** Which sequence strictly outlines the general process of RNA-directed gene silencing?
(A) dsRNA formation → Dicer processing → siRNA/RISC complex → Target mRNA recognition → mRNA cleavage
(B) Target mRNA recognition → dsRNA formation → siRNA/RISC complex → Dicer processing → mRNA cleavage
(C) Dicer processing → dsRNA formation → Target mRNA recognition → siRNA/RISC complex → mRNA cleavage
(D) dsRNA formation → Target mRNA recognition → Dicer processing → siRNA/RISC complex → mRNA cleavage

2017

**242.** What inference is valid regarding a crop plant extensively modified via gene pyramiding for pest resistance?

dissociation of $RF_1$ or RF2 from the ribosome after the polypeptide is released. RF3 does not directly recognize or bind to stop codons in the mRNA.

**27.** (A) Degrading ubiquitinated misfolded or short-lived proteins
The proteasome is a large, ATP-dependent multi-protein complex responsible for the regulated degradation of intracellular proteins. It specifically targets proteins that have been tagged with polyubiquitin chains. This pathway is crucial for removing misfolded or damaged proteins and for controlling the levels of short-lived regulatory proteins, such as those involved in cell cycle progression, signal transduction, and the stress response.

**28.** (C) It bends the DNA near the promoter to enhance RNA polymerase recruitment.
The cAMP-CAP complex acts as a positive regulator of the lac operon. When glucose levels are low, cAMP concentrations rise and bind to the Catabolite Activator Protein (CAP). This complex binds to a specific DNA site upstream of the promoter, inducing a sharp bend in the DNA. This structural change facilitates the recruitment and binding of RNA polymerase, significantly increasing transcription.

**29.** (D) Binding of the Trp repressor to the operator
Attenuation in the trp operon is controlled by the formation of alternative mRNA secondary structures in the leader sequence. This process depends on the speed of the ribosome, which is determined by the availability of charged tRNATrp. While the Trp repressor regulates transcription initiation at the operator, attenuation is a separate post-initiation mechanism governed by the coupling of transcription and translation dynamics.

**30.** (C) I, II and V
Epigenetic regulation involves modifications that affect gene expression without changing the DNA sequence. DNA methylation typically leads to gene silencing. Histone acetylation neutralizes the positive charge on lysine residues, weakening the interaction between histones and DNA to promote an open chromatin state. The histone code hypothesis suggests these modifications serve as specific binding sites for effector proteins that further regulate transcription.

**31.** (B) They use a free guanosine cofactor to initiate self-splicing.
Group I introns are self-splicing ribozymes that catalyze their own removal from RNA transcripts without the need for a spliceosome. The splicing reaction is initiated by a free guanosine nucleotide or nucleoside which acts as an external nucleophile. This guanosine binds to a specific site on the intron and attacks the 5' splice site, triggering a series of transesterification reactions.

**32.** (D) siRNAs are endogenous to all plants, whereas miRNAs are strictly introduced by viruses.
Both siRNAs and miRNAs are small RNA molecules involved in gene silencing. While they share the RISC machinery, their origins differ. MicroRNAs are endogenous molecules transcribed from the host genome and are essential for developmental regulation. In contrast, siRNAs can be either endogenous or exogenous, often derived from viral double-stranded RNA. The statement that miRNAs are strictly viral is an incorrect exception.

**33.** (A) It cleaves long double-stranded RNA into short 21-23 nucleotide fragments.
Dicer is a critical ribonuclease III enzyme in the RNA interference pathway. It recognizes and processes long double-stranded RNA or hairpin-shaped pre-miRNAs into short, double-stranded fragments approximately 21 to 23 nucleotides in length. These small RNAs are then loaded into the RISC complex to guide the sequence-specific silencing of target mRNAs, either through cleavage or translational inhibition.

**34.** (C) It facilitates iron and zinc chelation and transport within the plant.
Nicotianamine synthase is a key enzyme in the biosynthesis of nicotianamine, a natural chelator found in plants. Nicotianamine binds to metal ions like iron and zinc, facilitating their movement through the phloem to various tissues, including the seeds. Enhancing the expression of this gene is a strategic approach in biofortification to increase the accumulation and bioavailability of essential minerals in crops.

**35.** (A) Both A and R are true and R is the correct explanation of A.
Gene pyramiding involves incorporating multiple resistance genes targeting the same pathogen into a single plant variety. This strategy is more durable because a pathogen would need to acquire

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# ANSWERS

**1.** (A) Relaxed complementary base pairing at the third position of the codon
The wobble hypothesis explains how the translational machinery accommodates the redundancy of the genetic code. It proposes that the base pairing between the third position of the mRNA codon and the first position of the tRNA anticodon is less spatially constrained. This allows a single tRNA to recognize multiple synonymous codons, thereby reducing the total number of required tRNAs.

**2.** (C) They possess a Rossmann fold and attach the amino acid to the terminal 2' OH.
Aminoacyl-tRNA synthetases are categorized into two classes based on their structural motifs and catalytic mechanisms. Class I enzymes are characterized by the presence of a Rossmann fold active site. These enzymes typically catalyze the attachment of the specific amino acid to the 2' hydroxyl group of the terminal adenosine on the tRNA molecule, unlike Class II enzymes.

**3.** (A) 50S and 30S subunits containing 23S, 5S, and 16S rRNAs
Prokaryotic ribosomes are 70S complexes formed by a large 50S subunit and a small 30S subunit. The 50S subunit is composed of 23S and 5S ribosomal RNAs along with numerous proteins. The 30S subunit contains the 16S rRNA. These components work together to coordinate mRNA decoding and peptide bond formation during the process of protein synthesis.

**4.** (D) IF-2 is a GTPase that facilitates fMet-tRNAfMet binding to the P site.
Translation initiation in prokaryotes involves specific initiation factors that assemble the ribosomal complex. IF-2 is a specialized GTPase that recognizes and binds to the formylmethionyl-tRNA. It ensures the delivery of this initiator tRNA specifically to the ribosomal P site. This action is essential for establishing the correct reading frame before the large ribosomal subunit joins the complex.

**5.** (B) The initiator tRNA binds directly to the A site before scanning begins.
Eukaryotic translation initiation requires the assembly of the 43S pre-initiation complex, where the initiator methionyl-tRNA is positioned in the ribosomal P site. The complex binds to the 5' cap and scans the mRNA for the start codon. Binding to the A site is incorrect, as this site is reserved for incoming aminoacyl-tRNAs during the subsequent elongation phase of translation.

**6.** (A) Both A and R are true and R is the correct explanation of A.
EF-Tu is an elongation factor that carries aminoacyl-tRNA to the ribosomal A site. It protects the aminoacyl bond from hydrolysis and prevents premature peptide bond formation. This inhibition is achieved by physically masking the amino acid. Only upon correct codon-anticodon pairing does EF-Tu hydrolyze GTP and release the tRNA, ensuring high fidelity during the protein synthesis process.

**7.** (D) $eRF_1$
Translation termination in prokaryotes is mediated by release factors $RF_1$, RF2, and RF3, which recognize stop codons and facilitate the release of the polypeptide chain. $RF_1$ and RF2 recognize specific stop sequences, while RF3 assists their dissociation. In contrast, $eRF_1$ is the primary release factor in eukaryotic cells and is not involved in bacterial protein synthesis termination.

**8.** (A) It mimics aminoacyl-tRNA and causes termination by entering the A site.
Puromycin acts as a structural analog of the 3' end of an aminoacyl-tRNA molecule. During translation, it enters the ribosomal A site and reacts with the peptidyl-tRNA to form a peptidyl-puromycin linkage. Because this molecule cannot undergo translocation, the nascent polypeptide chain is prematurely released. This mechanism effectively inhibits protein synthesis in both prokaryotic and eukaryotic cells during elongation.

NODIA

(A) Completely blocking the synthesis of all iron-binding proteins in the root system.
(B) Synthesizing a totally artificial iron-based chlorophyll.
(C) Removing all traces of zinc to prevent competitive binding.
(D) Enhancing the production of the phytosiderophore nicotianamine, which strongly chelates iron and facilitates its transport and storage in edible tissues.

2023

**213.** Which concept application accurately reflects the purpose of stacking Bt toxins with different modes of action via gene pyramiding?
(A) To increase the nutritional quality of the crop
(B) To dramatically delay the evolution of resistance in target insect populations
(C) To make the crop completely resistant to broad-spectrum herbicides
(D) To increase the crop's natural drought tolerance

2009

**214.** Which statement about the Hepatitis Δ Virus (HDV) ribozyme is correct?
(A) It requires a massive spliceosome complex to fold properly.
(B) It acts as a powerful DNA ligase.
(C) It functions in vivo strictly to process the circular RNA genome of the virus during replication.
(D) It actively synthesizes the viral envelope proteins.

2010

**215.** Given below are two statements, one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The genetic code is considered universally conserved with very few exceptions.
Reason (R): The standard assignments of the 64 codons apply consistently across the vast majority of prokaryotic and eukaryotic nuclear genomes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**216.** Which of the following is the most relevant requirement for the Wobble hypothesis to hold true?
(A) Spatial flexibility at the 5' position of the anticodon (which pairs with the 3' position of the codon)
(B) Perfect rigid conformation of the entire anticodon loop
(C) Constant presence of inosine at the first position of the codon
(D) Absolute exclusion of modified bases in the tRNA

2021

**217.** Identify the correct process describing the interaction between a chaperone and its target.
(A) The chaperone binds covalently to the newly synthesized protein.
(B) The chaperone utilizes cycles of ATP binding and hydrolysis to alternately bind and release exposed hydrophobic segments, preventing premature aggregation.
(C) The chaperone completely engulfs the protein and heavily methylates its core.
(D) The chaperone strictly degrades any protein that fails to fold within one second.

2003

**218.** What defines the functional difference between an enhancer and a promoter?
(A) A promoter strictly regulates alternative splicing, while an enhancer regulates translation.
(B) A promoter precisely directs the initiation of transcription, whereas an enhancer amplifies the rate of transcription independent of precise distance or orientation.
(C) An enhancer requires entirely different RNA polymerases than a promoter.
(D) An enhancer is always transcribed, while a promoter is always translated.

2008

**219.** Given below are certain statements concerning protein degradation pathways.
I. Polyubiquitination targets proteins to the 26S proteasome.
II. Lysosomes primarily degrade extracellular or cell-surface proteins via endocytosis/ phagocytosis.
III. The proteasome heavily relies on ATP for unfolding its target substrates.
IV. Ubiquitin is structurally a highly variable, heavily glycosylated lipid.

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**18.** (A) Both A and R are true and R is the correct explanation of A.
Adenosine-to-inosine (A-to-I) RNA editing is a site-specific modification catalyzed by ADAR enzymes. Inosine is biologically recognized as guanosine by the translational machinery because it forms base pairs with cytosine. Consequently, this change can alter the codon sequence in the mRNA, leading to the incorporation of a different amino acid and thus changing the primary structure and function of the protein.

**19.** (B) dsRNA entry → Dicer cleavage → RISC assembly → Target mRNA cleavage
The siRNA pathway begins when long double-stranded RNA enters the cell and is recognized by the enzyme Dicer. Dicer cleaves the RNA into small interfering RNAs. These fragments are loaded into the RNA-Induced Silencing Complex (RISC). The antisense strand then guides the complex to a complementary mRNA target, which is cleaved by the Argonaute protein, resulting in gene silencing.

**20.** (D) I and III
Metagenomics is a powerful tool for bioprospecting that involves analyzing the total genetic material from environmental samples. This approach bypasses the need to culture individual microorganisms, which is particularly useful for discovering genes from extreme environments. It facilitates the identification of novel bioactive molecules and enzymes. Phenotypic screening and 16S rRNA amplification alone do not define the full scope of metagenomic gene discovery.

**21.** (B) Insertion of the phytoene synthase and carotene desaturase genes
Golden Rice is engineered to produce β-carotene in the rice endosperm to address vitamin A deficiency. This biofortification strategy involves introducing genes that complete the carotenoid biosynthetic pathway. Specifically, the insertion of phytoene synthase from plants and carotene desaturase from bacteria allows the conversion of geranylgeranyl diphosphate into β-carotene. This metabolic engineering bypasses the natural absence of these enzymes in rice grains.

**22.** (C) Marker-assisted selection using locus-specific markers.
Gene pyramiding involves combining multiple resistance genes into a single plant variety to provide durable protection against pathogens. Because individual genes often produce similar phenotypes, visual selection is insufficient to confirm their presence. Marker-assisted selection uses DNA-based markers closely linked to each gene of interest. This allows breeders to precisely identify and track the inheritance of multiple alleles throughout the breeding program.

**23.** (D) GFP - Real-time in vivo sub-cellular localization
Green Fluorescent Protein (GFP) is a widely used reporter tool in molecular biology. When fused to a target protein, it allows researchers to visualize the protein's real-time distribution and movement within living cells using fluorescence microscopy. Unlike GUS, which usually requires tissue fixation and substrate addition, GFP fluorescence is intrinsic and non-destructive, making it ideal for studying dynamic cellular processes in vivo.

**24.** (A) A small catalytic RNA motif comprising three base-paired helices converging on a core
The hammerhead ribozyme is a small, naturally occurring RNA molecule with intrinsic catalytic activity. Its structure is defined by three base-paired helices that converge on a highly conserved central core of non-helical nucleotides. This arrangement allows the ribozyme to catalyze site-specific cleavage of the RNA backbone via a transesterification reaction. It is a classic model for studying RNA-mediated catalysis and structure-function relationships.

**25.** (B) P-4, Q-3, R-2, S-1
Antibiotics and toxins target protein synthesis through various mechanisms. Tetracycline binds the 30S subunit A-site, blocking tRNA entry. Chloramphenicol inhibits the 50S peptidyl transferase center. Cycloheximide targets the eukaryotic 60S peptidyl transferase. Diphtheria toxin inactivates eukaryotic eEF2 by catalyzing its ADP-ribosylation. These compounds are essential tools for distinguishing prokaryotic and eukaryotic translational processes and are used in both research and medicine.

NODIA

**26.** (B) RF3 - Recognizes the UAA codon directly in prokaryotes
In prokaryotic translation termination, stop codons are recognized by Class I release factors. $RF_1$ recognizes UAA and UAG, while RF2 identifies UAA and UGA. RF3 is a Class II release factor and a GTPase that facilitates the

(A) It will definitely suffer from severe yield drag under all conditions.
(B) It will inherently become highly toxic to human consumption.
(C) It is significantly less likely to experience a rapid breakdown in resistance compared to a monoculture expressing a single resistance trait.
(D) It relies entirely on continuous chemical pesticide application.

2024

**243.** Identify the correct pair describing a translational process and its precise location.
(A) Codon-anticodon recognition - 40S subunit decoding center (A-site)
(B) Peptidyl transfer - 40S subunit P-site
(C) mRNA scanning - 60S subunit E-site
(D) Exit of uncharged tRNA - 40S subunit A-site

2022

**244.** Which is an advantage of employing a His-tag rather than a GST-tag for protein purification?
(A) The His-tag inherently massively increases the solubility of totally insoluble proteins.
(B) The His-tag specifically drives the protein directly into the cell nucleus.
(C) The His-tag avoids the absolute need for any type of affinity chromatography.
(D) The His-tag is smaller (typically 6-10 amino acids) and is much less likely to interfere with the target protein's structure or function.

2017

**245.** What defines the specific role of IF-1 during prokaryotic translation initiation?
(A) It actively forms the critical peptide bond.
(B) It binds to the 30S A-site to prevent premature entry of the initiator tRNA, forcing it into the P-site.
(C) It fundamentally hydrolyzes GTP to lock the subunits together.
(D) It heavily phosphorylates the 16S rRNA.

2012

**246.** Which point accurately characterizes the Kozak consensus sequence?
(A) It consists entirely of purely poly-uracil tracts.
(B) It strictly requires exactly ten perfectly matched complementary nucleotides.
(C) It relies predominantly on the specific spacing between the $-3$ and $+4$ positions relative to the AUG start codon.
(D) It operates exclusively in extremely deep-sea archaebacteria.

2008

**247.** Which cause and effect relationship correctly characterizes protein phosphorylation?
(A) Phosphatase activity → Addition of phosphate group → Rapid degradation → Loss of function
(B) Kinase activity → Removal of phosphate group → Increased hydrophobic interactions → Aggregation
(C) Phosphatase activity → Removal of specific lipid anchors → Translocation to the nucleus → Enhanced transcription
(D) Kinase activity → Addition of bulky negatively charged phosphate group → Conformational change → Altered protein activity

2011

**248.** What is the function of the signal peptidase?
(A) It specifically cleaves the N-terminal signal sequence from the growing polypeptide chain after it enters the ER lumen.
(B) It synthesizes the highly hydrophobic signal peptide.
(C) It actively forces the nascent polypeptide through the Sec61 translocon.
(D) It degrades the entire signal recognition particle (SRP) after targeting.

2009

**249.** Which concept application best describes the utility of identifying novel promoter elements via plant bioprospecting?
(A) Generating entirely sterile plant varieties.
(B) Developing precisely regulated transgenic crops that express stress-resistance traits only when challenged by that specific stress.
(C) Permanently suppressing all natural photosynthetic activity.
(D) Forcing the plant to exclusively utilize anaerobic respiration pathways.

2015

**250.** What is the most appropriate overarching principle of the "Wobble" base pairing?
(A) It demonstrates that the genetic code is highly unstable and prone to failure.
(B) It enables physiological efficiency, allowing a cell to translate all 61 sense codons using fewer than 61 distinct tRNA species.
(C) It strictly limits the evolutionary adaptability of viral genomes.
(D) It proves that the first two positions of the codon are biologically completely irrelevant.

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several specific, simultaneous mutations to overcome all the stacked resistance mechanisms. Mathematically, the probability of such multiple mutations occurring at once is extremely low, thus preventing the rapid breakdown of crop resistance.

**36.** (D) Helix-loop-helix
The helix-loop-helix (HLH) motif is a structural domain characterized by two α helices connected by a flexible, non-helical loop. This motif is frequently found in transcription factors where it mediates protein dimerization. The helices allow the proteins to form homodimers or heterodimers, which is essential for their ability to bind DNA and regulate the expression of specific target genes.

**37.** (B) A specific internal adenosine forming a 2'-5' phosphodiester bond
Group II introns are self-splicing RNAs that utilize a mechanism similar to the nuclear spliceosome. The splicing process is initiated by an internal nucleophile, specifically the 2' hydroxyl group of a conserved adenosine residue within the intron. This attacks the 5' splice site, creating a lariat intermediate characterized by a unique 2'-5' phosphodiester bond. This autocatalytic process does not require ATP.

**38.** (C) Cleavage of pri-miRNA to pre-miRNA in the nucleus
Drosha is a Class II ribonuclease III enzyme located in the nucleus. It is a central component of the Microprocessor complex, which processes long primary microRNA transcripts (pri-miRNAs). Drosha cleaves these pri-miRNAs into hairpin-shaped precursor microRNAs (pre-miRNAs) approximately 60 to 70 nucleotides long. These pre-miRNAs are then exported to the cytoplasm for further processing by Dicer into mature microRNAs.

**39.** (D) Deamination by specific cytidine deaminase enzymes
RNA editing in the mitochondria and chloroplasts of plants frequently involves the conversion of cytidine to uridine. This site-specific modification is catalyzed by cytidine deaminase enzymes. The reaction involves the removal of an amino group from the cytosine base, transforming it into uracil. This process can significantly alter the coding sequence of the transcript, often restoring conserved amino acid sequences necessary for function.

**40.** (A) ER → Golgi → Secretory Vesicle → Plasma Membrane
The secretory pathway in eukaryotic cells follows a highly organized sequence for protein transport. Synthesis begins on ribosomes attached to the rough endoplasmic reticulum (ER). Modified proteins are then transported via COPII-coated vesicles to the Golgi apparatus for further processing and sorting. Finally, they are packaged into secretory vesicles that fuse with the plasma membrane to release the contents externally.

**41.** (B) Small catalytic RNA involved in RNA genome cleavage and ligation
The Hepatitis Δ Virus (HDV) ribozyme is a small, self-cleaving RNA motif found within the viral genome. It is essential for the rolling-circle replication of the virus, where it catalyzes the site-specific cleavage of multimeric RNA transcripts into individual genomic units. This ribozyme is unique among nucleolytic ribozymes for its high stability and its functional requirement for activity within a human host.

**42.** (B) Presence of highly diverse allelic variations for stress tolerance
Wild relatives of cultivated crops serve as invaluable genetic reservoirs for plant breeding. During the process of domestication, many alleles associated with resilience to biotic and abiotic stresses were lost in favor of high yield and uniformity. Wild species often retain diverse allelic variations that confer resistance to drought, salinity, and pests, making them primary targets for bioprospecting and modern crop improvement.

**43.** (A) His-tag - Purified using immobilized metal affinity chromatography (IMAC)
Affinity-tag fusion systems are essential for the purification of recombinant proteins. A polyhistidine tag (His-tag) coordinates with divalent metal ions like nickel or cobalt. This allows the fusion protein to be captured using immobilized metal affinity chromatography (IMAC). Other systems like GST use glutathione agarose, while Maltose-Binding Protein uses amylose resin. These tags simplify purification and can often be removed using specific proteases.

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**77.** (D) Phosphorylation
Phosphorylation is one of the most common and versatile post-translational modifications. It involves the addition of a negatively charged phosphate group to the hydroxyl group of serine, threonine, or tyrosine residues by enzymes called kinases. This modification can significantly alter a protein's conformation, activity, and interactions. In transcription factors, phosphorylation often regulates their ability to bind DNA or enter the nucleus, providing a rapid switch for gene expression.

**78.** (C) An N-terminal hydrophobic signal peptide
The destination of a newly synthesized protein is largely determined by specific signal sequences within its primary structure. Proteins destined for the secretory pathway typically possess an N-terminal signal peptide characterized by a core of hydrophobic amino acids. This sequence is recognized by the signal recognition particle (SRP) during translation, which halts synthesis and directs the ribosome to the endoplasmic reticulum membrane for co-translational translocation into the lumen.

**79.** (B) It is a gratuitous inducer that binds the repressor but is not metabolized.
Isopropyl β-D-1-thiogalactopyranoside (IPTG) is a molecular mimic of allolactose used in laboratory settings to induce the lac operon. It is called a "gratuitous inducer" because it binds effectively to the lac repressor, causing it to detach from the operator, but it cannot be cleaved by β-galactosidase. Consequently, IPTG concentrations remain constant during an experiment, providing a stable and predictable induction of recombinant protein expression in bacterial cultures.

**80.** (A) Enhancer → TATA Box → Transcription Start Site → Translation Start Codon
The regulatory regions of a typical eukaryotic gene are organized in a specific sequence to coordinate transcription and translation. Enhancers are often located upstream and modulate the rate of transcription. The TATA box is a core promoter element that helps position RNA polymerase II near the transcription start site. Downstream of the transcription start is the 5' untranslated region, which ends at the AUG translation start codon.

NODIA

**81.** (A) Trp operon attenuation
Attenuation is a regulatory mechanism in the tryptophan operon that utilizes alternative RNA secondary structures. Depending on tryptophan levels, the leader mRNA can form either an antiterminator or a terminator stem-loop. The terminator structure, composed of regions 3 and 4, forces RNA polymerase to dissociate before transcribing the structural genes. This system allows the cell to rapidly adjust gene expression based on the real-time availability of charged tryptophan tRNAs.

**82.** (C) They are transcribed into the final coding region of the mRNA.
Enhancers are cis-acting DNA sequences that increase the transcriptional efficiency of a gene. They are characterized by their ability to function from great distances and in either orientation. Unlike exons, enhancers are regulatory elements and are not transcribed into the mature mRNA or translated into the protein. They serve as platforms for the assembly of transcription factor complexes that interact with the promoter through DNA looping.

**83.** (A) The associated gene is likely transcriptionally silenced.
CpG islands are genomic regions with a high frequency of cytosine-guanine dinucleotides, often located near gene promoters. Heavy methylation of these cytosines is a strong epigenetic signal for transcriptional silencing. The methyl groups interfere with the binding of transcription factors and recruit proteins that promote the formation of repressive heterochromatin. Therefore, a highly methylated CpG island is a reliable indicator that the adjacent gene is not being expressed.

**84.** (D) Splicing of the first intron to supply the cap structure
The formation of the 5' cap is a co-transcriptional process that occurs shortly after the start of RNA synthesis. It involves three enzymatic steps: removal of the 5' terminal phosphate, addition of a guanine nucleotide in an unusual 5'-to-5' linkage, and methylation of the guanine at the N7 position. This process is entirely independent of splicing. The cap is essential for mRNA stability, nuclear export, and recognition by the ribosome.

ribozyme function. If this center is inhibited, the ribosome cannot transfer the growing polypeptide chain from the P-site tRNA to the incoming aminoacyl-tRNA in the A-site. Consequently, the elongation phase of translation is halted.

**53.** (B) It recognizes the stop codons UAA and UAG.
In Escherichia coli, translation termination is triggered when a stop codon enters the ribosomal A-site. This signal is recognized by Class I release factors. $RF_1$ specifically identifies the stop codons UAA and UAG. Upon binding, $RF_1$ promotes the hydrolysis of the ester bond between the polypeptide and the tRNA, leading to the release of the completed protein from the translational machinery.

**54.** (C) It targets the hydroxyl oxygen of serine or threonine residues.
O-linked glycosylation is a post-translational modification where sugar molecules are attached to the oxygen atom of the hydroxyl group on serine or threonine residues. Unlike N-linked glycosylation, which begins in the ER with a large pre-assembled oligosaccharide, O-linked glycosylation typically occurs in the Golgi apparatus. It involves the sequential addition of individual sugars and does not require a dolichol lipid carrier.

**55.** (A) Both A and R are true and R is the correct explanation of A.
The ubiquitin-proteasome pathway is an energy-dependent process for protein degradation. ATP hydrolysis is required at multiple steps, starting with the activation of ubiquitin by the E1 enzyme. This enzyme uses ATP to create a high-energy thioester bond with ubiquitin, enabling its subsequent transfer to E2 and E3 enzymes. Additionally, the 26S proteasome requires ATP to unfold target proteins before they enter the catalytic core.

**56.** (A) Nuclear localized transcription factors
The classical secretory pathway involves proteins synthesized on the rough ER, which are then transported through the Golgi to lysosomes, the plasma membrane, or the extracellular space. In contrast, nuclear localized transcription factors are synthesized on free ribosomes in the cytoplasm. They are subsequently imported into the nucleus through nuclear pore complexes using specific nuclear localization signals, bypassing the endoplasmic reticulum and Golgi transport.

**57.** (B) LacA - Lactose permease
The lac operon consists of structural genes and regulatory elements. LacZ encodes β-galactosidase, which cleaves lactose. LacY encodes lactose permease, which facilitates the entry of lactose into the cell. LacA encodes a transacetylase whose exact role in lactose metabolism is less clear. LacI is the repressor protein that binds the operator (LacO) sequence. Identifying LacA as the permease is an incorrect pairing in this context.

**58.** (B) Ribosome stalling at consecutive Trp codons in the leader sequence
Attenuation in the trp operon depends on the intracellular levels of tryptophan. When tryptophan is scarce, the ribosome stalls at two consecutive Trp codons in the leader sequence. This stalling prevents region 1 from pairing with region 2, allowing regions 2 and 3 to form an antiterminator hairpin. This structure prevents the formation of the terminator hairpin (3-4 pairing), allowing RNA polymerase to continue transcription.

**59.** (A) Transcriptional activation
Histone acetylation is a key epigenetic modification associated with transcriptionally active chromatin, known as euchromatin. Enzymes called histone acetyltransferases add acetyl groups to lysine residues on histone tails, neutralizing their positive charge. This reduces the electrostatic attraction between histones and the negatively charged DNA backbone, leading to a more relaxed chromatin structure. This open configuration facilitates the binding of transcription factors and RNA polymerase.

**60.** (C) I, III and IV
The 3' polyadenylation of eukaryotic mRNA involves the addition of a tail of adenine nucleotides. This process requires a conserved AAUAAA consensus sequence for recognition. Unlike primary transcription, the poly-A tail is added by poly(A) polymerase without a DNA template. This modification is crucial for protecting the mRNA from exonucleolytic degradation, assisting in nuclear export, and significantly enhancing the efficiency of translation in the cytoplasm.

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**61.** (D) The 5' splice site will not be properly recognized.
Pre-mRNA splicing by the spliceosome begins with the recognition of specific sequences. The U1 small nuclear ribonucleoprotein (snRNP) contains an RNA component that is complementary to the conserved 5' splice site. Binding of U1 to this site is the initial step in defining the intron-exon boundaries. If a transcript cannot bind U1 snRNP, the 5' splice site will not be identified, preventing the subsequent assembly of the spliceosome.

**62.** (C) Post-transcriptional alteration of the nucleotide sequence of an RNA transcript
RNA editing refers to various molecular processes that change the nucleotide sequence of an RNA molecule after it has been transcribed from the DNA template. This can include the insertion, deletion, or substitution of individual bases. Such modifications can alter the meaning of codons, create new start or stop signals, or affect splicing. RNA editing is particularly prevalent in the organelles of plants and certain animal tissues.

**63.** (B) By guiding the RISC complex to cleave perfectly complementary target mRNA
Small interfering RNAs (siRNAs) mediate post-transcriptional gene silencing by targeting specific messenger RNAs for degradation. After being processed by Dicer, siRNAs are loaded into the RNA-Induced Silencing Complex (RISC). The guide strand of the siRNA provides the specificity required to find a perfectly complementary sequence on the target mRNA. Once bound, the catalytic component of RISC cleaves the mRNA, preventing its translation into a protein.

**64.** (D) Function-driven screening of expression libraries for specific enzymatic activity
Metagenomics allows for the discovery of novel genes from environmental samples. Sequence-driven screening is useful when looking for known gene families, but function-driven screening is superior for finding entirely new enzymes. This approach involves creating an expression library where environmental DNA fragments are expressed in a host like E. coli. The library is then screened for specific phenotypic traits or biochemical activities, regardless of the gene's sequence.

**65.** (A) P-2, Q-3, R-1, S-4
Transgenic biofortification targets various nutrients using specific metabolic components. Phytoene synthase is the key enzyme for provitamin A (β-carotene) synthesis. Ferritin serves as a storage protein for iron. Nicotianamine synthase produces a chelator that facilitates the transport and accumulation of zinc and iron. Amaranth seed albumin (AmA1) is used to improve the balance of essential amino acids in staple crops like potatoes and rice.

**66.** (C) It allows simultaneous selection of multiple genes with similar traits.
Marker-assisted gene pyramiding is a powerful breeding technique that uses DNA markers to identify the presence of multiple target genes. Unlike conventional phenotypic selection, which can be difficult when multiple genes produce the same observable trait, molecular markers allow breeders to confirm the presence of each specific allele. This enables the efficient stacking of resistance genes, providing more robust and durable protection against evolving pathogens.

**67.** (A) It can sometimes aggregate or cause steric hindrance when fused to certain target proteins.
While Green Fluorescent Protein (GFP) is an invaluable tool for live-cell imaging, it has some limitations. As a relatively large protein, GFP can occasionally interfere with the natural folding, localization, or biological activity of the protein to which it is fused. In some cases, high levels of expression can lead to protein aggregation. These potential artifacts must be carefully considered when interpreting results from fusion protein experiments.

NODIA

**68.** (D) Two independently folding domains (loops A and B) that dock to form the active site
The hairpin ribozyme is a small catalytic RNA found in satellite RNAs of plant viruses. Its secondary structure consists of four helices and two internal loops, known as loops A and B. For catalysis to occur, these two independently folding domains must dock together to form a specific three-dimensional active site. This ribozyme catalyzes site-specific cleavage and ligation reactions essential for the replication of the viral RNA.

**69.** (B) Organisms prefer specific synonymous codons matching tRNA abundances.
Codon usage bias refers to the non-random use of synonymous codons to encode the same amino acid. Organisms often show a preference for specific codons that correspond to the most abundant isoaccepting tRNAs in the cell. This preference is particularly evident in highly expressed genes, where it serves to optimize the speed and accuracy of translation. Such bias is a reflection of evolutionary selection for translational efficiency.

**70.** (C) I, II and V
The tRNA molecule possesses a characteristic cloverleaf secondary structure that folds into a tertiary L-shape. The D loop is named for its dihydrouridine residues, while the TΨC loop contains pseudouridine. These modified bases are essential for the structural stability and function of the tRNA. The tertiary structure is stabilized by complex base-pairing and stacking interactions between these loops, ensuring the proper positioning of the anticodon and acceptor stems.

**71.** (D) P-site - Anchors the growing peptidyl-tRNA chain
The ribosome contains three distinct sites for tRNA binding during elongation. The A (aminoacyl) site receives incoming charged tRNAs. The P (peptidyl) site holds the tRNA attached to the growing polypeptide chain. The E (exit) site is where uncharged tRNAs are positioned before they are released from the ribosome. These sites ensure the orderly progression of the mRNA and the coordinated synthesis of the peptide chain.

**72.** (A) A ribozyme integral to the large ribosomal subunit
Peptidyl transferase is the central enzymatic activity of the ribosome, responsible for forming peptide bonds between amino acids. Research has demonstrated that this activity is not catalyzed by a protein, but rather by the large ribosomal RNA molecule (23S in prokaryotes and 28S in eukaryotes). This classifies the ribosome as a ribozyme, highlighting the fundamental role of RNA in both the storage of genetic information and catalysis.

**73.** (B) It delivers the initiator Met-tRNAi to the 40S subunit in a GTP-dependent manner.
eIF2 is a critical eukaryotic initiation factor that forms a ternary complex with GTP and the initiator methionyl-tRNA. This complex binds to the 40S ribosomal subunit to form the 43S pre-initiation complex. The hydrolysis of GTP by eIF2 is a key regulatory step that occurs after the start codon is recognized, facilitating the release of initiation factors and the subsequent joining of the 60S subunit.

**74.** (B) Action of the Ribosome Recycling Factor (RRF) alongside EF-G and GTP hydrolysis
After translation termination and the release of the polypeptide, the ribosome remains bound to the mRNA and uncharged tRNA. Ribosome recycling is the process of disassembling this post-termination complex. In bacteria, this is achieved by the concerted action of Ribosome Recycling Factor (RRF) and Elongation Factor G (EF-G). Powered by GTP hydrolysis, these factors promote the dissociation of the ribosomal subunits, allowing them to be reused.

**75.** (A) Both A and R are true and R is the correct explanation of A.
Diphtheria toxin is a potent inhibitor of eukaryotic protein synthesis. It enters the cell and acts as an enzyme that catalyzes the covalent attachment of an ADP-ribose group to elongation factor eEF2. This modification specifically targets a modified histidine residue called diphthamide. The ADP-ribosylation of eEF2 permanently blocks its ability to facilitate ribosomal translocation along the mRNA, leading to the rapid cessation of translation and cell death.

**76.** (C) They permanently integrate into the final structure of the folded protein.
Molecular chaperones are essential proteins that assist others in reaching their native, functional three-dimensional conformations. They work by binding to exposed hydrophobic patches on unfolded or partially folded polypeptides, preventing inappropriate aggregation. Chaperones typically function through cycles of ATP binding and hydrolysis. Importantly, they are transient assistants; they do not become part of the final protein structure and are recycled to assist other newly synthesized polypeptides.

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intron as a lariat and ligate the adjacent exons together to form the mature mRNA.

**109.** (A) Pri-miRNA → Pre-miRNA → Mature miRNA
The biogenesis of microRNA follows a specific sequential pathway. It begins with the transcription of long primary microRNAs (pri-miRNAs) from the genome. In the nucleus, the Drosha enzyme processes these into hairpin-shaped precursor microRNAs (pre-miRNAs). After export to the cytoplasm, the Dicer enzyme further cleaves the pre-miRNA into a small double-stranded RNA duplex. One strand of this duplex is then loaded into the RISC complex to become the mature, functional miRNA.

**110.** (C) I, III and IV
Post-transcriptional gene silencing (PTGS) is a sequence-specific mechanism for regulating mRNA stability. It requires homology between a small guide RNA and its target transcript. The process is mediated by the RISC complex, which contains Argonaute proteins as the catalytic engine. In plants, PTGS can be systemic, as the silencing signal can spread through the organism via plasmodesmata. This allows the plant to coordinate a widespread response to viral infections or developmental cues.

**111.** (B) It is strictly limited to traits already present within the crop's natural gene pool.
Transgenic biofortification is a powerful approach because it can introduce entirely new biosynthetic pathways into a crop. Unlike traditional breeding, which is restricted to the genetic variation existing within sexually compatible species, transgenics can utilize genes from bacteria, fungi, or unrelated plants. This flexibility allows researchers to produce essential micronutrients, like provitamin A in Golden Rice, even when the natural gene pool of the crop lacks the necessary enzymatic capabilities.

**112.** (D) The probability of insects developing resistance to all three toxins simultaneously is mathematically extremely low.
Gene pyramiding involves stacking multiple defense genes, such as different Bt toxins, into a single crop variety. The primary advantage of this strategy is the delay of pest resistance. For an insect to survive on a pyramided plant, it would need to possess mutations that confer resistance to all three toxins at the same time. The mathematical probability of such multiple, independent mutations occurring simultaneously in a single insect is exceptionally low, thereby extending the utility of the resistant crop.

**113.** (A) Fusing a GFP tag to a protein allows for its visual tracking within a living cell.
Gene fusion is a technique where the coding sequences of two different proteins are joined to create a single chimeric molecule. A classic example is fusing a target protein to Green Fluorescent Protein (GFP). This creates a functional fusion protein that retains the biological properties of the target while being fluorescent. This allows researchers to track the protein's real-time localization and movement within live cells using microscopy, providing insights into its cellular function and dynamics.

**114.** (C) An RNA molecule capable of acting as an enzyme to catalyze specific biochemical reactions
A ribozyme is a specialized RNA molecule that possesses intrinsic catalytic activity. This discovery fundamentally changed the understanding of biological catalysis, which was previously thought to be exclusive to proteins. Ribozymes use their complex three-dimensional structures to facilitate specific biochemical reactions, such as the cleavage or ligation of RNA backbones. Examples include the peptidyl transferase center of the ribosome and self-splicing introns, highlighting the ancient and essential roles of RNA in life.

**115.** (A) tRNA - Cloverleaf and L-shape
Transfer RNA (tRNA) molecules exhibit highly conserved structural motifs essential for their role in translation. The secondary structure is typically represented as a cloverleaf, featuring an acceptor stem, a D loop, an anticodon loop, and a TΨC loop. This cloverleaf folds into a compact, three-dimensional L-shaped tertiary structure. This specific shape is critical because it precisely positions the anticodon and the attached amino acid to interact with the ribosome and the mRNA during protein synthesis.

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**116.** (D) It allows a single tRNA to recognize multiple synonymous codons, reducing tRNA requirements.
The wobble position, located at the first base of the tRNA anticodon and the third base of the mRNA codon, allows for non-standard base pairing. This flexibility is a key feature of the

**85.** (B) P-4, Q-1, R-3, S-2
Pre-mRNA splicing and self-splicing involve distinct recognition events and nucleophiles. U1 snRNP binds to the 5' splice site, while U2 snRNP recognizes the branch point sequence. Group I introns use an external guanosine as a nucleophile to initiate the reaction. Group II introns, like the spliceosome, utilize an internal adenosine nucleophile to form a lariat intermediate. These mechanisms illustrate the diverse pathways used to remove non-coding sequences from RNA transcripts.

**86.** (C) It involves ADAR enzymes acting on double-stranded RNA regions.
Adenosine-to-inosine (A-to-I) RNA editing is a prevalent modification in eukaryotic transcripts. It is catalyzed by Adenosine Deaminases acting on RNA (ADARs), which target specific double-stranded RNA structures. The enzyme removes an amino group from adenosine, converting it to inosine. Since inosine base-pairs with cytosine, it is read as guanosine during translation. This can lead to amino acid substitutions, altered splicing, or changes in RNA stability and localization.

**87.** (D) Argonaute - Cleaves the target mRNA within the RISC complex
The RNA interference pathway involves several specialized proteins. Drosha and Dicer are ribonucleases that process long RNA precursors into small RNAs. Argonaute is the central catalytic component of the RNA-Induced Silencing Complex (RISC). When the guide RNA is perfectly complementary to its target mRNA, Argonaute cleaves the transcript, leading to gene silencing. This sequence-specific degradation is a fundamental mechanism for regulating gene expression and defending against viral infections.

**88.** (A) They possess traits like drought resistance lost during crop domestication.
Wild relatives of crops are vital resources for plant bioprospecting because they have evolved under natural selection in diverse environments. During domestication, many genes conferring tolerance to environmental stresses like drought, extreme temperatures, and local pests were lost as breeders focused on high yield and uniformity. Wild relatives retain these resilient alleles, which can be identified and reintroduced into modern varieties through breeding or genetic engineering to improve crop sustainability.

**89.** (B) Introduction of genes bridging the gap from GGPP to β-carotene.
Rice endosperm naturally produces geranylgeranyl diphosphate (GGPP), a precursor for carotenoids, but lacks the enzymes to convert it into β-carotene. The biofortification strategy used in Golden Rice involves introducing the genes for phytoene synthase and carotene desaturase. These enzymes bridge the metabolic gap, allowing the rice grain to synthesize and accumulate β-carotene. This transgenic approach successfully converts a staple crop into a source of provitamin A to combat nutritional deficiencies.

**90.** (B) I and II
Gene pyramiding is the strategy of combining multiple resistance genes into a single variety. It is achieved through systematic crossing, backcrossing, and the use of molecular markers to track the genes. By presenting multiple defense mechanisms, this approach reduces the selection pressure on pathogens, as they are less likely to overcome all resistance factors at once. This results in more durable crop protection compared to the deployment of single resistance genes.

**91.** (A) They alter the fundamental genetic code to incorporate non-natural amino acids.
Affinity-tag fusion systems are powerful tools for recombinant protein production. By genetically fusing a specific tag, such as a His-tag or GST-tag, to a target protein, researchers can use affinity chromatography for rapid, single-step purification. These tags are composed of standard amino acids and do not require changes to the universal genetic code. Often, a protease cleavage site is included to allow the removal of the tag after the purification process is complete.

**92.** (C) It catalyzes a reversible transesterification reaction yielding a 2',3'-cyclic phosphate and a 5'-hydroxyl.
The hairpin ribozyme is a small catalytic RNA found in certain plant viruses. It facilitates the processing of viral RNA by catalyzing a site-specific transesterification reaction. This reaction cleaves the phosphodiester backbone, producing a 2',3'-cyclic phosphate and a 5'-hydroxyl group. Importantly, the reaction is reversible, allowing the ribozyme to also catalyze the ligation of RNA fragments. Its activity depends entirely on its specific three-dimensional folding and does not require protein subunits.

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**93.** (D) The non-random usage of synonymous codons during translation
Codon usage bias refers to the observation that synonymous codons are not used with equal frequency in a genome. Different organisms show preferences for specific codons that match the abundance of their corresponding tRNAs. This bias is most pronounced in genes with high expression levels, where it serves to maximize the efficiency and accuracy of the translational machinery. Understanding this bias is crucial for optimizing gene expression in heterologous hosts.

**94.** (C) Binding to the minor groove of the tRNA acceptor stem and utilizing a Rossmann fold
Class I aminoacyl-tRNA synthetases are distinguished by several structural and functional features. They possess a characteristic Rossmann fold active site and typically interact with the minor groove of the tRNA acceptor stem. During the aminoacylation reaction, they first attach the amino acid to the 2' hydroxyl group of the terminal adenosine. In contrast, Class II enzymes use a β-sheet fold, bind the major groove, and target the 3' hydroxyl group.

**95.** (A) Both A and R are true and R is the correct explanation of A.
The 16S rRNA is a fundamental component of the prokaryotic 30S ribosomal subunit and plays a vital role in translation initiation. Its 3' terminus contains a sequence known as the anti-Shine-Dalgarno sequence. This region base-pairs with the complementary Shine-Dalgarno sequence located in the 5' untranslated region of the mRNA. This interaction is essential for accurately positioning the start codon in the ribosomal P-site to ensure correct translation.

**96.** (D) It allows the design of expression vectors that maximize translation efficiency in eukaryotic systems.
The Kozak consensus sequence is a motif surrounding the AUG start codon that influences the efficiency of translation initiation in eukaryotes. By understanding which nucleotides are preferred at specific positions, particularly the -3 and +4 positions, researchers can optimize synthetic genes. Incorporating a strong Kozak sequence into expression vectors ensures that the ribosome efficiently recognizes the start site, leading to higher levels of protein production in transfected or transgenic eukaryotic cells.

**97.** (A) It escorts the aminoacyl-tRNA to the ribosomal A-site in a GTP-dependent manner.
$eEF_1A$ is a central eukaryotic elongation factor that functions as a GTPase. Its primary role is to form a complex with GTP and an incoming aminoacyl-tRNA. This ternary complex delivers the tRNA to the ribosomal A-site. Only when the tRNA anticodon correctly pairs with the mRNA codon does $eEF_1A$ hydrolyze GTP and release the tRNA. This mechanism ensures that only the correct amino acids are incorporated into the growing polypeptide chain.

**98.** (C) eEF2
In both prokaryotes and eukaryotes, the elongation phase of translation requires a specialized factor to promote ribosomal translocation. In bacteria, this role is performed by Elongation Factor G (EF-G). Its functional analog in eukaryotic cells is eEF2. Both are GTPases that use the energy from GTP hydrolysis to drive the movement of the ribosome along the mRNA by exactly three nucleotides, shifting the tRNAs from the A and P sites to the P and E sites.

**99.** (A) The coordinate action of RRF, EF-G, and IF3
After translation termination, the 70S ribosome remains associated with the mRNA and a deacylated tRNA. Disassembly and recycling of the ribosome are essential for subsequent rounds of synthesis. This process requires the Ribosome Recycling Factor (RRF) and Elongation Factor G (EF-G), which use GTP hydrolysis to dissociate the subunits. Initiation Factor 3 (IF3) then binds to the 30S subunit, preventing it from prematurely rejoining the 50S subunit until a new initiation complex is formed.

**100.** (D) I and II
Translation inhibitors are valuable tools for studying protein synthesis. Streptomycin binds to the prokaryotic 30S subunit and causes misreading of the genetic code. Cycloheximide inhibits the peptidyl transferase activity specifically in eukaryotic 60S subunits. Chloramphenicol is specific for prokaryotic 50S subunits and mitochondria. Puromycin mimics aminoacyl-tRNA and acts as a chain terminator in both systems, rather than an inhibitor of RNA polymerase. These differences allow for selective inhibition in various cell types.

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**101.** (B) Polyubiquitinated proteins
The ubiquitin-proteasome system is the primary pathway for regulated protein degradation in eukaryotes. Proteins destined for destruction are covalently tagged with a chain of ubiquitin molecules. This polyubiquitin chain serves as a specific recognition signal for the 26S proteasome. The proteasome complex captures these tagged proteins, unfolds them in an ATP-dependent manner, and translocates them into its interior catalytic core where they are hydrolyzed into small peptides for recycling.

**102.** (C) N-linked occurs on Asparagine; O-linked occurs on Serine/Threonine.
Protein glycosylation is categorized by the specific amino acid residue to which the carbohydrate is attached. N-linked glycosylation involves the attachment of an oligosaccharide to the amide nitrogen of an asparagine residue, typically occurring co-translationally in the ER. O-linked glycosylation involves the attachment of sugars to the oxygen atom of the hydroxyl group on serine or threonine residues. This process usually occurs post-translationally in the Golgi apparatus and follows a different biosynthetic pathway.

**103.** (D) Addition of initial en bloc oligosaccharide to Asparagine
The Golgi apparatus is a central hub for protein modification and sorting. It is the site for O-linked glycosylation and the complex pruning and remodeling of N-linked oligosaccharides. However, the initial addition of the "en bloc" 14-sugar oligosaccharide to asparagine residues occurs exclusively in the endoplasmic reticulum. This core glycosylation is a co-translational event, and the resulting glycoprotein is only later transported to the Golgi for further specialized modifications.

**104.** (A) Inducing recombinant protein expression under the control of the lac promoter
IPTG is a non-metabolizable analog of lactose used extensively in molecular biology. It functions as a powerful inducer by binding to the lac repressor protein, causing it to undergo a conformational change and detach from the operator DNA. This allows RNA polymerase to transcribe the downstream genes. In cloning, IPTG is used to trigger the high-level expression of recombinant proteins from vectors that utilize the lac or related promoter systems.

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**105.** (B) P-3, Q-4, R-2, S-1
Eukaryotic gene regulation involves various DNA elements and protein factors. Silencers bind repressor proteins to downregulate transcription. Promoters contain essential core elements like the TATA box and initiator sequences to position RNA polymerase. General transcription factors are required for basal initiation at these promoters. Specific transcription factors bind to distal enhancers to modulate the rate of transcription, providing a highly sophisticated system for controlling gene expression across different cell types.

**106.** (B) Steric hindrance of transcription factor binding and recruitment of histone deacetylases
DNA methylation at promoter regions is a robust epigenetic mechanism for gene silencing. The presence of methyl groups on cytosines can physically block the binding of many transcription factors to their target sites. Furthermore, methylated DNA recruits methyl-binding proteins that serve as scaffolds for histone deacetylases. These enzymes remove acetyl groups from histones, promoting the formation of compact heterochromatin, which further restricts the access of the transcriptional machinery to the gene.

**107.** (A) It protects the mRNA from 5'-to-3' exonucleases and assists in ribosome binding.
The 5' cap is a critical modification of eukaryotic messenger RNA. It consists of a 7-methylguanosine residue linked to the transcript via a unique 5'-to-5' triphosphate bridge. This structure serves two primary functions: it shields the mRNA from degradation by 5' exonucleases, thereby increasing its half-life, and it provides a vital recognition signal for the eIF4F complex. This interaction is essential for the efficient recruitment of the ribosome during translation initiation.

**108.** (C) It utilizes snRNAs to recognize splice sites and catalyze two transesterification reactions.
The spliceosome is a large ribonucleoprotein complex responsible for removing introns from pre-mRNA. It is composed of five small nuclear RNAs (snRNAs) and numerous proteins. The snRNAs recognize the 5' splice site, the 3' splice site, and the branch point through base pairing. The spliceosome then coordinates two sequential transesterification reactions that excise the

**44.** (C) Palmitoylation irreversibly attaches a lipid strictly via an amide linkage.
Post-translational lipid modifications increase the hydrophobicity of proteins, facilitating membrane association. Myristoylation involves a stable amide bond at the N-terminus, while prenylation attaches isoprenoids to a C-terminal cysteine. Palmitoylation, however, is unique because it involves a reversible thioester linkage between a palmitoyl group and an internal cysteine residue. This reversibility allows cells to dynamically regulate the membrane localization and signaling of specific proteins.

**45.** (D) P-3, Q-2, R-4, S-1
Transcription factors utilize diverse structural domains for DNA binding and dimerization. Zinc fingers use coordinated metal ions for stability. Leucine zippers facilitate dimerization through interdigitating hydrophobic residues. The helix-turn-helix motif is a classic bacterial DNA-binding structure found in the Lac repressor. The homeodomain is a highly conserved 60-amino acid motif characteristic of Hox genes, which play vital roles in developmental patterning.

**46.** (C) A cis-acting DNA sequence that stimulates transcription from a distance.
Enhancers are regulatory DNA elements that significantly increase the transcription of target genes. They are cis-acting and characterized by their ability to function over long distances, regardless of their orientation or position relative to the promoter. Enhancers serve as binding sites for transcriptional activators, which interact with the basal transcription machinery through DNA looping, often mediated by the Mediator complex.

**47.** (B) It requires the substrate X-Gluc, producing a blue precipitate in fixed tissues.
The GUS reporter system, based on the β-glucuronidase enzyme, is a powerful tool for analyzing gene expression in plants. When the substrate X-Gluc is applied to plant tissues expressing the GUS gene, the enzyme catalyzes a reaction that produces a stable blue precipitate. This histochemical assay allows researchers to identify the specific tissues and developmental stages where a promoter is active.

**48.** (D) A single amino acid can be encoded by more than one synonymous codon.
The genetic code is described as degenerate because most amino acids are specified by multiple synonymous codons. There are 61 sense codons that encode only 20 standard amino acids. This redundancy often occurs at the third position of the codon. Degeneracy provides a buffer against the harmful effects of mutations, as a change in the DNA sequence may not always alter the protein.

**49.** (A) A distinct editing domain that hydrolyzes mischarged amino acids from the tRNA
Aminoacyl-tRNA synthetases ensure translational fidelity through a two-step mechanism. After the initial activation of the amino acid, the enzyme checks the match. If an incorrect, typically smaller, amino acid is attached to the tRNA, it is moved to a separate editing domain. This domain hydrolyzes the ester bond, releasing the incorrect amino acid and preventing the production of erroneous proteins by the ribosome.

**50.** (C) I and III
The 80S eukaryotic ribosome consists of a 60S large subunit and a 40S small subunit. The 60S subunit contains the 28S, 5.8S, and 5S rRNAs, while the 40S subunit contains the 18S rRNA. Ribosomal assembly is a complex process that occurs primarily within the nucleolus. Unlike prokaryotes, eukaryotes do not use Shine-Dalgarno sequences; instead, they typically utilize a 5' cap-dependent scanning mechanism.

**51.** (A) It acts as a scaffold linking the 5' cap complex to the 40S subunit.
eIF4G is a large scaffolding protein that plays a central role in eukaryotic translation initiation. It is a component of the eIF4F complex, where it coordinates multiple interactions. It binds to the cap-binding protein eIF4E and the poly-A binding protein, circularizing the mRNA. Additionally, eIF4G interacts with eIF3, facilitating the recruitment of the 40S ribosomal subunit to the mRNA transcript.

**52.** (D) Cessation of translation elongation due to lack of peptide bond formation
The peptidyl transferase center, located within the 23S rRNA of the large ribosomal subunit in prokaryotes, is responsible for catalyzing peptide bond formation. This enzymatic activity is a

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genetic code's degeneracy. It enables a single tRNA species to successfully pair with multiple synonymous codons that differ only at their third position. Consequently, cells can efficiently translate all 61 sense codons using a significantly smaller number of distinct tRNA molecules.

**117.** (B) Both utilize RNA for peptidyl transfer, but the catalytic rRNA is 23S in prokaryotes and 28S in eukaryotes.
Both prokaryotic and eukaryotic ribosomes are ribozymes that use large ribosomal RNA to catalyze peptide bond formation. In the prokaryotic 70S ribosome, this activity is centered in the 23S rRNA of the 50S subunit. In the eukaryotic 80S ribosome, it is located in the 28S rRNA of the 60S subunit. While the specific rRNA species differ, the fundamental mechanism of peptidyl transfer is conserved across all domains of life.

**118.** (C) Enhancing the efficiency of translational start site recognition in eukaryotic expression systems
The Kozak sequence is a specific nucleotide motif surrounding the start codon that is preferred by the eukaryotic translational machinery. In synthetic biology and genetic engineering, optimizing this sequence in an expression vector ensures that the 40S ribosomal subunit correctly and efficiently identifies the initiation site. This optimization is a common strategy to maximize the production of recombinant proteins in eukaryotic cells by ensuring high levels of translation initiation from the transcript.

**119.** (D) It binds to the 30S subunit to prevent premature association with the 50S subunit.
Initiation Factor 3 (IF-3) is a vital component of the prokaryotic translational machinery. Its primary role is to bind to the small 30S ribosomal subunit. This binding serves two critical purposes: it prevents the 30S subunit from prematurely re-associating with the 50S subunit, and it facilitates the correct binding of the mRNA and the initiator tRNA. IF-3 ensures that the 30S initiation complex is fully and accurately assembled before translation begins.

**120.** (A) I, II, III, IV
Eukaryotic translation elongation follows a precise cyclic sequence. First, $eEF_1A$ complexed with GTP delivers the appropriate aminoacyl-tRNA to the ribosomal A-site. Once the tRNA is accommodated, the peptidyl transferase activity of the large subunit catalyzes the formation of a peptide bond. Subsequently, eEF2 uses the energy from GTP hydrolysis to promote the translocation of the ribosome along the mRNA. Finally, the uncharged tRNA is moved to and released from the E-site.

**121.** (B) It recognizes all three stop codons in eukaryotes and triggers peptide release.
$eRF_1$ is the primary Class I release factor in eukaryotic translation termination. Unlike prokaryotes, which use two distinct release factors to recognize different stop codons, eukaryotes utilize a single factor, $eRF_1$, to identify all three termination signals: UAA, UAG, and UGA. Upon binding to the stop codon in the ribosomal A-site, $eRF_1$ promotes the hydrolysis of the bond between the polypeptide and the tRNA, leading to the successful release of the completed protein.

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**122.** (B) They depurinate a specific universally conserved adenine in the 28S rRNA, halting elongation.
Ricin is a potent toxin that acts as a Ribosome-Inactivating Protein (RIP). It functions as a highly specific N-glycosidase that targets a universally conserved adenine residue within the sarcin-ricin loop of the eukaryotic 28S ribosomal RNA. By removing this single adenine base, ricin permanently disrupts the ability of the ribosome to interact with elongation factors. This irreversible modification causes a complete and rapid halt in protein synthesis, leading to the death of the affected cell.

**123.** (A) It primarily folds proteins before they are translated by the ribosome.
The GroEL-GroES system is a classic bacterial chaperonin complex that assists in protein folding. GroEL forms a double-ring structure that creates two isolated chambers, while GroES acts as a lid. This complex functions post-translationally, capturing polypeptides after they have been synthesized by the ribosome. The chamber provides a sequestered environment that prevents aggregation while the protein folds into its native state. This process is energy-dependent and requires cycles of ATP binding and hydrolysis.

**124.** (C) Reversible protein phosphorylation
Protein phosphorylation is a major regulatory mechanism that controls a vast array of cellular

processes, including signal transduction and the cell cycle. The addition of a negatively charged phosphate group by kinases and its removal by phosphatases act as a rapid molecular switch. This modification can activate or deactivate proteins and change their localization. Because it is highly dynamic and reversible, phosphorylation allows cells to respond quickly and precisely to internal signals and external environmental changes.

**125.** (A) Rough ER → COPII vesicles → Golgi → Secretory vesicles
Proteins destined for secretion follow a defined pathway through the endomembrane system. Synthesis begins on the rough endoplasmic reticulum (ER), where the proteins are folded and initially modified. They are then packaged into COPII-coated vesicles for transport to the Golgi apparatus. In the Golgi, they undergo further processing and sorting. Finally, the mature proteins are loaded into secretory vesicles that move toward and fuse with the plasma membrane to release their contents externally.

**126.** (C) The operon will be constitutively expressed, provided glucose is absent (and cAMP is high).
The lac operon is normally under negative control by the LacI repressor. In a mutant lacking a functional repressor (LacI-), the operon is no longer inhibited and becomes constitutively expressed. However, the operon is also subject to catabolite repression. Even without the repressor, high levels of transcription still require the cAMP-CAP complex, which only forms when glucose is absent. Therefore, maximal expression occurs continuously as long as glucose levels do not trigger catabolite repression.

**127.** (B) Low tryptophan → Ribosome stalls at Trp codons → Antiterminator forms → Transcription continues
Attenuation in the trp operon is governed by the availability of tryptophan. When tryptophan levels are low, the ribosome stalls at two consecutive Trp codons in the leader sequence. This stalling physically prevents region 1 from pairing with region 2, which allows regions 2 and 3 to form a stable antiterminator hairpin. This structure prevents the formation of the downstream terminator, allowing RNA polymerase to proceed through the structural genes and complete the transcription process.

**128.** (D) Core promoter - Determines the exact initiation site of transcription
Eukaryotic gene regulation relies on specific DNA elements. The core promoter is the region typically located near the transcription start site. It contains essential elements like the TATA box and initiator, which serve as the assembly platform for the general transcription factors and RNA polymerase II. By organizing these components, the core promoter precisely determines the location and direction of transcription initiation, establishing the foundation for all subsequent gene expression and regulation.

**129.** (A) They utilize ATP hydrolysis to slide, eject, or restructure nucleosomes, altering DNA accessibility.
ATP-dependent chromatin remodeling complexes are essential for regulating gene expression. They use the energy from ATP hydrolysis to physically move, remove, or alter the composition of nucleosomes along the DNA. This structural dynamic is critical because it controls the accessibility of the underlying DNA sequence to the transcriptional machinery. By exposing or masking promoters and enhancers, these complexes play a central role in both the activation and repression of target genes.

**130.** (D) Dictating the correct reading frame for the ribosome
The 3' poly-A tail of eukaryotic mRNA serves multiple essential roles in the cell. It protects the transcript from 3'-to-5' exonucleolytic degradation, facilitates its export from the nucleus to the cytoplasm, and enhances translational efficiency through its interaction with poly-A binding proteins and initiation factors. However, the poly-A tail is located after the stop codon and does not play a role in determining the reading frame, which is established at the AUG start codon.

**131.** (A) A branched RNA molecule featuring a 2'-5' phosphodiester bond at the branch point adenosine
During pre-mRNA splicing, the excision of an intron proceeds through two transesterification steps. In the first step, the 2' hydroxyl group of a conserved internal adenosine, known as the branch point, attacks the 5' splice site. This reaction creates a characteristic lariat structure where the 5' end of the intron is covalently linked to the branch point adenosine via a 2'-

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5' phosphodiester bond. This branched RNA intermediate is later released and degraded.

**132.** (D) Post-transcriptional alteration of the mRNA coding sequence
RNA editing in plants often involves the site-specific deamination of cytidine to uridine. This process is catalyzed by specialized cytidine deaminase enzymes. Because it occurs after transcription but before translation, it is a post-transcriptional mechanism that can change the sequence of the mRNA. This can lead to amino acid substitutions in the encoded protein, often restoring conserved residues that are essential for the protein's biological function within the mitochondria or chloroplasts.

**133.** (B) It primarily functions in the nucleus to inhibit transcription.
The RNA-Induced Silencing Complex (RISC) is the central executioner of post-transcriptional gene silencing in the cytoplasm. It incorporates a small single-stranded guide RNA (siRNA or miRNA) and a catalytic Argonaute protein. The RISC uses the guide RNA to identify complementary mRNA targets. Depending on the degree of complementarity, the complex then induces the degradation of the target mRNA or inhibits its translation, thereby effectively regulating gene expression at the post-transcriptional level.

**134.** (C) It allows access to the genetic material of unculturable microbes for novel gene discovery.
Metagenomics is particularly powerful because it estimates that over 99% of microorganisms in nature cannot be grown under standard laboratory conditions. By directly extracting and analyzing DNA from environmental samples, researchers can bypass the culturing requirement and access the "dark matter" of the microbial world. This provides a massive genetic reservoir for the discovery of novel enzymes, antibiotics, and bioactive molecules that were previously hidden and inaccessible to traditional microbiological methods.

**135.** (A) Both A and R are true and R is the correct explanation of A.
Transgenic metabolic engineering is a highly effective strategy for biofortification. It allows for the precise introduction of multiple genes required to reconstruct entire biochemical pathways in specific tissues. For example, in Golden Rice, the insertion of several genes enables the synthesis of provitamin A in the endosperm, which naturally lacks this pathway. This provides a sustainable and cost-effective method to continuously enrich staple crops with essential micronutrients for local populations.

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**136.** (A) Robust molecular markers linked to each target gene must be available for accurate selection.
Successful gene pyramiding requires the ability to precisely track multiple genes as they are combined into a single variety. Because multiple genes for the same trait (like pest resistance) often produce identical phenotypes, visual selection is impossible. Therefore, robust molecular markers that are tightly linked to each individual target gene are essential. These markers allow breeders to use DNA-based screening to confirm that each specific gene has been successfully inherited in the offspring.

**137.** (B) It allows single-step affinity purification of the fusion protein using a glutathione-sepharose column.
The Glutathione S-transferase (GST) tag is a commonly used fusion system for recombinant protein purification. GST has a high and specific affinity for its substrate, glutathione. By genetically fusing GST to a target protein, the resulting fusion protein can be selectively captured from a complex cell lysate using a glutathione-sepharose affinity column. This allows for rapid, single-step purification. The bound protein can then be gently eluted using a buffer containing free reduced glutathione.

**138.** (B) They possess intrinsic catalytic activity strictly dependent on their RNA tertiary structure.
Ribozymes are a unique class of RNA molecules that function as biological catalysts. Their enzymatic activity is derived from their ability to fold into complex, stable three-dimensional structures, similar to protein enzymes. These tertiary structures create specific active sites that can coordinate metal ions and facilitate chemical reactions, most commonly the cleavage or ligation of phosphodiester bonds. This intrinsic catalytic capability demonstrates that RNA can act as both genetic material and a functional enzyme.

**154.** (B) It generates multiple distinct mature mRNAs and protein isoforms from a single primary transcript.
Alternative splicing is a fundamental mechanism that enhances the complexity of eukaryotic gene expression. By selectively including or excluding different exons during the processing of a primary pre-mRNA transcript, a single gene can produce a variety of mature mRNA molecules. Each of these transcripts can be translated into a distinct protein isoform with unique functional or structural properties. This process significantly expands the diversity of the cellular proteome without requiring an increase in genome size.

**155.** (A) Both A and R are true and R is the correct explanation of A.
RNA interference mediated by siRNAs is a classic example of post-transcriptional gene silencing (PTGS). This is because the mechanism targets mRNA molecules that have already been fully transcribed and processed. The siRNAs are loaded into the RISC complex, which then uses the small RNA as a guide to locate and cleave perfectly complementary target mRNAs in the cytoplasm. By destroying the transcripts before they can be translated, the cell effectively silences the corresponding gene.

**156.** (C) It can correct disease-causing point mutations at the transcript level without permanent genomic modification.
Targeted RNA editing using artificial deaminases offers a significant therapeutic advantage over traditional gene editing. By modifying the RNA transcript rather than the underlying DNA, it is possible to correct genetic errors without introducing permanent changes to the host genome. This approach is inherently safer as it avoids the risks of off-target genomic mutations. Furthermore, the effects are transient and can be controlled, providing a flexible and powerful tool for treating a variety of genetic diseases.

**157.** (D) It catalyzes site-specific cleavage of its target RNA via a transesterification reaction.
The hammerhead ribozyme is a small catalytic RNA molecule characterized by its ability to perform self-cleavage. It folds into a specific three-dimensional structure that creates an active site for catalysis. The ribozyme promotes a site-specific transesterification reaction that breaks the phosphodiester backbone of the RNA. Because this activity is intrinsic to the RNA molecule itself and does not require protein enzymes, it serves as a fundamental model for understanding RNA-mediated catalysis in nature and biotechnology.

**158.** (C) They allow for the simultaneous integration of an entire targeted metabolic pathway (e.g., biofortification) in a single transformation event.
Multi-gene vectors are sophisticated tools in plant biotechnology designed to express several different proteins from a single construct. This approach is particularly valuable for complex metabolic engineering projects, such as biofortification, where multiple enzymes are needed to complete a new biochemical pathway. By delivering all the necessary genes in one transformation event, researchers can ensure their co-inheritance and coordinated expression, greatly simplifying the development of transgenic varieties with enhanced nutritional profiles.

**159.** (B) They require the presence of strong detergents to bind to the affinity column.
Histidine tags (His-tags) are a popular affinity fusion system consisting of six to ten consecutive histidine residues. They work by coordinating with divalent metal ions like nickel or cobalt immobilized on a chromatographic resin. This binding is highly specific and occurs under both native and denaturing conditions. His-tags do not require detergents for binding; in fact, the bound proteins are typically eluted using imidazole, which competes for the metal coordination sites, allowing for efficient and gentle purification.

**160.** (D) I, II and IV
A functional reporter construct requires several essential genetic elements to ensure proper expression and detection. A promoter is necessary to initiate transcription at the correct site. An uninterrupted open reading frame of the reporter gene, such as GFP or GUS, is required to produce a functional protein. Finally, a transcriptional terminator is essential to signal the end of transcription and ensure mRNA stability. A eukaryotic origin of replication is typically not required for standard genomic integration and expression.

**161.** (A) The cloverleaf represents 2D secondary structure, while the L-shape represents the 3D tertiary structure.
The structure of a tRNA molecule is described at multiple levels. The cloverleaf is a two-

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bacteria by stopping their ability to synthesize essential proteins.

**147.** (A) Ubiquitination (poly) - Targeted protein degradation
Post-translational modifications are essential for regulating protein function and fate. The attachment of a polyubiquitin chain to a lysine residue is a universal signal for targeted degradation in eukaryotic cells. This modification is recognized by the 26S proteasome, which unfolds and breaks down the tagged protein into small peptides. This pathway is critical for removing misfolded proteins and for controlling the levels of key regulatory molecules, such as cyclins and transcription factors.

**148.** (D) All nuclear-encoded mitochondrial proteins utilize the exact same ER-targeting signal peptide.
The signal peptide hypothesis explains how proteins are targeted to the endoplasmic reticulum for secretion. While most secretory proteins use hydrophobic N-terminal signals, targeting to other organelles like mitochondria follows different rules. Mitochondrial proteins are synthesized in the cytoplasm and typically possess distinct, positively charged amphipathic presequences that target them directly to the mitochondrial translocon (TOM/TIM). Therefore, they do not use the ER-targeting pathway, representing a significant exception to the general secretory signal peptide mechanism.

**149.** (B) It is likely a nucleic-acid binding protein or transcription factor.
Zinc finger domains are one of the most common structural motifs used for DNA and RNA recognition. These domains consist of small protein folds stabilized by the coordination of one or more zinc ions. Due to their specific geometry, they can fit precisely into the major groove of the DNA double helix. Therefore, the presence of a zinc finger domain is a strong structural indicator that the protein functions as a transcription factor or another nucleic acid-binding regulator.

**150.** (C) The physical binding of the LacI repressor to the operator sequence
The lac operon is primarily controlled through a mechanism of negative regulation. In the absence of lactose, the LacI repressor protein is synthesized and binds specifically to the operator DNA sequence. This binding creates a physical barrier that prevents RNA polymerase from moving through the structural genes, effectively silencing transcription. Only when an inducer molecule like allolactose binds to the repressor does it change shape and detach, allowing the gene expression to proceed.

**151.** (D) It involves the formation of alternative RNA secondary structures depending on translation speed.
Attenuation is a sensitive regulatory mechanism found in several bacterial operons, including the trp operon. It functions through the coupling of transcription and translation in the leader sequence of the mRNA. The speed at which a ribosome moves through this sequence, which depends on nutrient levels, determines which of several mutually exclusive RNA stem-loop structures will form. One of these structures acts as a transcriptional terminator, providing an immediate and efficient response to cellular conditions.

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**152.** (A) Two $\alpha$ helices forming a coiled-coil dimer via periodic leucine residues
The leucine zipper is a common structural motif found in many eukaryotic transcription factors. It is characterized by an $\alpha$ helix containing a leucine residue at every seventh position. These regularly spaced hydrophobic residues allow two such helices to interdigitate, forming a stable coiled-coil dimer. This dimerization is essential for the proteins to bind to their target DNA sequences effectively. The motif provides a versatile platform for the formation of both homodimers and heterodimers.

**153.** (B) Specific combinations of post-translational histone modifications dictate chromatin structure and transcriptional states.
The "histone code" hypothesis proposes that the diverse post-translational modifications of histone tails, such as acetylation, methylation, and phosphorylation, serve as a complex regulatory language. Specific combinations of these marks are recognized by "reader" proteins that subsequently alter chromatin structure or recruit the transcriptional machinery. This system allows for the sophisticated and hereditary control of gene expression, providing a layer of information that extends beyond the primary DNA sequence itself.

nascent chain complex to the SRP receptor on the rough endoplasmic reticulum membrane. This mechanism ensures that proteins destined for secretion or membrane insertion are correctly delivered to the translocon.

**169.** (B) High glucose → Low cAMP → CAP does not bind promoter → Transcription is severely reduced
Catabolite repression is a mechanism that allows bacteria like E. coli to prioritize the use of glucose over other sugars. When glucose is abundant, the levels of cAMP in the cell are low. Consequently, the Catabolite Activator Protein (CAP) cannot bind cAMP to form an active complex. Without the active cAMP-CAP complex binding upstream of the promoter to assist RNA polymerase recruitment, the transcription of the lac operon remains at a very low basal level, even if lactose is present.

**170.** (C) Ribosome translates quickly → Region 2 is blocked → Region 3 pairs with 4 → Terminator forms
Attenuation in the trp operon is driven by the speed of the ribosome relative to the transcription of the leader sequence. When tryptophan is abundant, the ribosome moves quickly through the leader sequence and covers region 2. This prevents region 2 from base-pairing with region 3. As a result, region 3 is free to pair with the newly transcribed region 4, forming a stable terminator stem-loop. This structure causes RNA polymerase to dissociate, terminating transcription before the structural genes.

**171.** (A) Their functional capability strictly relies on their orientation relative to the promoter.
Enhancers are key regulatory DNA elements characterized by their unique properties. They serve as clustered binding sites for various transcription factors and can exert their influence over very large genomic distances by looping to the promoter. Importantly, unlike many other genetic elements, enhancers are orientation-independent. This means they can stimulate the transcription of their target gene regardless of whether they are located in a forward or reverse direction relative to the promoter.

**172.** (A) It acts as a molecular bridge between distal enhancers and the core promoter complex.
The Mediator complex is a massive, multi-subunit coactivator essential for eukaryotic transcription. Its primary role is to serve as a functional bridge between distal regulatory elements, such as enhancers, and the general transcription machinery assembled at the core promoter. By coordinating signals from multiple transcription factors and facilitating the recruitment and activity of RNA polymerase II, Mediator integrates complex regulatory inputs to ensure the precise timing and level of gene expression in various cellular contexts.

**173.** (D) They recognize splice sites via RNA-RNA base pairing and facilitate the catalytic splicing reactions.
Small nuclear ribonucleoproteins (snRNPs) are the core components of the eukaryotic spliceosome. Each snRNP consists of a small nuclear RNA and a set of specific proteins. The RNA components are essential because they use complementary base pairing to precisely recognize the 5' splice site, the 3' splice site, and the branch point in the pre-mRNA. This structural organization allows the snRNPs to coordinate and catalyze the complex transesterification reactions required to remove introns and join exons accurately.

**174.** (C) To generate distinct protein isoforms that provide variable resistance traits under different environmental stresses
In plant biotechnology, engineering alternative splicing pathways is a strategic approach to enhance crop resilience. By manipulating how a single gene is processed, researchers can produce multiple protein isoforms from one transcript. This is particularly useful for creating plants that can produce different versions of a defense or stress-response protein depending on environmental cues. Such flexibility allows the crop to adapt its physiology and resistance mechanisms dynamically, providing more robust protection against diverse abiotic and biotic stresses.

**175.** (A) Both A and R are true and R is the correct explanation of A.
Metagenomics involves analyzing the vast genetic diversity present in environmental samples. Because the majority of DNA fragments in these samples will not encode the specific enzyme or bioactive molecule of interest, functional screening is inherently a low-frequency event. To overcome this, high-throughput technologies are essential to rapidly screen millions of clones

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dimensional representation of its secondary structure, highlighting the four major base-paired stems and loops. In the cell, this cloverleaf folds further into a compact, three-dimensional L-shaped tertiary structure. This final shape is stabilized by interactions between the loops and is essential for the tRNA to fit precisely into the ribosomal A and P sites during the process of translation.

**162.** (C) IF-1 - Enhances IF-2 binding and stabilizes the 30S complex
Prokaryotic translation initiation is a highly regulated process involving three specific initiation factors. IF-1 plays a key role by binding to the A-site of the small 30S ribosomal subunit. This binding prevents the premature entry of tRNAs into the A-site and also enhances the affinity of the subunit for IF-2. By stabilizing the 30S initiation complex, IF-1 ensures that the initiator tRNA and mRNA are correctly positioned before the large ribosomal subunit joins.

**163.** (A) The 43S pre-initiation complex moves 5'-to-3' from the cap until it encounters the AUG start codon within a Kozak consensus.
Eukaryotic translation typically begins with a cap-dependent scanning mechanism. The 43S pre-initiation complex, which contains the small ribosomal subunit and the initiator tRNA, binds to the 5' end of the mRNA. Powered by ATP-dependent helicases, the complex then moves along the transcript in a 5'-to-3' direction. It "scans" the sequence until it identifies the first AUG start codon in an appropriate context, established by the Kozak consensus, to begin protein synthesis.

**164.** (D) It acts as a GEF, regenerating the active $eEF_1A$-GTP complex from $eEF_1A$-GDP.
In the eukaryotic elongation cycle, $eEF_1A$ is a GTPase that delivers aminoacyl-tRNAs to the ribosome. After successful delivery, $eEF_1A$ hydrolyzes GTP and is released as an inactive $eEF_1A$-GDP complex. To participate in another round of elongation, the GDP must be replaced with GTP. This nucleotide exchange is catalyzed by $eEF_1B$, which functions as a guanine nucleotide exchange factor (GEF). This regeneration step is vital for maintaining a continuous and efficient supply of active elongation factors.

**165.** (B) P-3, Q-4, R-1, S-2
Eukaryotic gene expression involves several specialized molecular factors. Dicer is the ribonuclease responsible for processing double-stranded RNA into siRNAs and miRNAs. The TATA-binding protein is a central component of the basal transcription machinery, identifying the promoter start site. eEF2 is the elongation factor that drives ribosomal translocation along the mRNA. $eRF_1$ is the release factor that recognizes stop codons to terminate translation. Each of these components performs a distinct and essential role in the pathway.

**166.** (C) Diphtheria toxin targets the elongation factor eEF2.
Many translational inhibitors specifically target the structural components of the ribosome. Chloramphenicol and erythromycin bind to the large 50S subunit of prokaryotes, while tetracycline targets the small 30S subunit. In contrast, Diphtheria toxin is an exception because its primary target is not the ribosome itself, but rather an accessory protein. It catalyzes the ADP-ribosylation of the eukaryotic elongation factor eEF2, permanently inactivating it and causing a complete halt in translocation and protein synthesis.

**167.** (D) It involves the covalent attachment of farnesyl or geranylgeranyl isoprenoid groups to a C-terminal cysteine.
Prenylation is a specific type of post-translational lipid modification that increases a protein's affinity for cellular membranes. It involves the attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid group. This covalent bond is typically formed with a cysteine residue located within a conserved CaaX motif at the C-terminus of the protein. Prenylation is essential for the proper localization and biological activity of many important signaling proteins, such as members of the Ras superfamily.

**168.** (A) A ribonucleoprotein complex that recognizes signal sequences and targets ribosomes to the rough ER
The signal recognition particle (SRP) is a universally conserved ribonucleoprotein complex that plays a vital role in protein trafficking. During translation, the SRP recognizes and binds to a hydrophobic signal peptide as it emerges from the ribosome. This binding temporarily pauses translation and targets the entire ribosome-

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5' linkages, the cap is formed via an unusual 5'-to-5' triphosphate bridge. This specific chemical arrangement is critical for protecting the mRNA from exonucleases and for providing a recognizable signal for the translational initiation machinery.

**183.** (D) Prokaryotes do not require GTP hydrolysis for the action of RF3.
Translation termination in prokaryotes is a multi-step process involving several release factors. $RF_1$ and RF2 recognize stop codons and catalyze the release of the polypeptide. Following this, RF3, which is a specialized GTPase, binds to the ribosome. The hydrolysis of GTP by RF3 is essential because it provides the energy required to promote the dissociation of the Class I release factor ($RF_1$ or RF2) from the ribosomal A-site, allowing the recycling of these factors for subsequent termination events.

**184.** (A) They bind transiently to exposed hydrophobic patches on non-native proteins to prevent aggregation.
Molecular chaperones like Hsp70 are essential for maintaining cellular protein quality. They act by recognizing and binding to exposed hydrophobic amino acid sequences that are typically buried in a natively folded protein. By transiently covering these patches, chaperones prevent non-specific and potentially harmful aggregation between unfolded or partially folded polypeptides. This assistance allows the proteins the time and environment necessary to fold correctly into their functional native states, particularly during the stress of synthesis or denaturation.

**185.** (B) P-3, Q-4, R-1, S-2
Post-translational modifications target specific amino acid residues to regulate protein function. N-linked glycosylation occurs on the amide nitrogen of asparagine. Phosphorylation is the addition of a phosphate group to the hydroxyl group of serine, threonine, or tyrosine. Acetylation typically targets the amino group of lysine residues. O-linked glycosylation involves the attachment of sugars to the hydroxyl oxygen of serine or threonine. These modifications are critical for protein stability, localization, and activity across all eukaryotic organisms.

**186.** (B) It allows the host cell to secrete the mature, processed recombinant protein into the culture medium, simplifying purification.
In recombinant protein production, engineering a signal peptide allows the host cell to direct the protein to the secretory pathway. As the protein enters the endoplasmic reticulum, the signal peptidase cleaves the signal sequence, yielding the mature protein. The cell subsequently secretes this processed protein into the extracellular culture medium. This is a major advantage for industrial production because it separates the target protein from most intracellular contaminants, significantly simplifying and reducing the cost of the downstream purification process.

**187.** (A) The rate of intrinsic transcription termination at the end of the lacA gene
The initiation frequency of the lac operon is primarily determined by regulatory events at the promoter and operator. These include the intracellular concentration of cAMP, which activates the CAP protein, and the binding affinity of the Lac repressor to the operator. The exact sequence of the core promoter also influences how well RNA polymerase binds. In contrast, the termination rate at the far end of the operon (after lacA) does not affect how often new rounds of transcription are initiated.

**188.** (C) It consists of two α helices, one of which specifically fits into the major groove of DNA.
The helix-turn-helix (HTH) motif is a classic structural domain found in many DNA-binding proteins, particularly in prokaryotic repressors. It consists of two α helices separated by a short, flexible turn. One of these helices, known as the recognition helix, contains specific amino acids that interact with the bases in the major groove of the DNA double helix. This allows the protein to recognize and bind to specific regulatory sequences with high affinity, controlling the transcription of target genes.

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**189.** (D) Methylated cytosines serve as binding sites for methyl-CpG-binding domain (MBD) proteins, which recruit histone deacetylases.
DNA methylation is a powerful epigenetic mechanism that leads to transcriptional silencing through a multi-step process. Cytosine methylation at CpG sites provides a specific recognition signal for Methyl-CpG-Binding Domain (MBD) proteins. Once

for the desired activity. This efficiency is critical for successfully identifying novel genes from unculturable microorganisms and turning complex metagenomic libraries into functional biological discoveries.

**176.** (D) I, II and IV
Golden Rice is a biofortified variety engineered to accumulate β-carotene in its starchy endosperm. This metabolic engineering requires the insertion of a phytoene synthase gene, often sourced from daffodil or maize, and a carotene desaturase gene from the bacterium Erwinia uredovora. These enzymes work together to reconstitute the carotenoid biosynthetic pathway, which is naturally inactive in rice grains. This provides a source of provitamin A in a staple food to help combat childhood blindness and other vitamin deficiencies.

**177.** (A) It enables the identification of plants carrying multiple resistance genes even when their individual phenotypes are indistinguishable.
Marker-assisted selection (MAS) is a cornerstone of modern gene pyramiding. When multiple resistance genes produce similar observable effects, traditional phenotypic screening cannot distinguish between a plant with one gene and a plant with several. MAS uses DNA-based markers to directly detect the presence of each specific allele in the genome. This allows breeders to efficiently stack multiple resistance genes into a single variety, creating more durable and robust protection against pathogens without the need for extensive, time-consuming bioassays.

**178.** (C) GUS (β-glucuronidase) - Commonly used for histochemical localization in plant tissues
Reporter genes are essential tools for studying gene expression. The GUS system is particularly popular in plant biology because it produces a stable blue precipitate when the enzyme reacts with the substrate X-Gluc. This allows for precise histochemical localization of promoter activity across different tissues and developmental stages. In contrast, GFP is used for live imaging, and luciferase requires specific cofactors for light emission. GUS remains a standard for definitive, high-resolution mapping of gene expression patterns in fixed plant materials.

**179.** (A) Mutations in the third codon position are less likely to result in an amino acid change (synonymous mutations).
The wobble hypothesis describes the flexible base-pairing rules at the third position of the codon. Because a single tRNA can recognize multiple codons that differ only at this site, many amino acids are encoded by synonymous codons. Consequently, a point mutation occurring at the third position of a codon is frequently a "silent" or synonymous mutation that does not alter the resulting amino acid sequence. This degeneracy provides a natural buffer against genetic damage and helps maintain the integrity of protein function.

**180.** (D) Specific recognition of the tRNA's anticodon loop and acceptor stem by its cognate aminoacyl-tRNA synthetase.
The accuracy of the genetic code depends on the high specificity of aminoacyl-tRNA synthetases. Each enzyme is designed to recognize a unique set of identity elements on its cognate tRNA, typically located in the anticodon loop and the acceptor stem. By using these structural cues, the enzyme ensures that it only attaches the correct amino acid to its corresponding tRNA. This precise matching is the "second genetic code" and is essential for maintaining translational fidelity during protein synthesis.

**181.** (B) It is the exit site where uncharged, deacylated tRNA temporarily binds before leaving the ribosome.
During the elongation phase of translation, the ribosome coordinates the movement of tRNAs through three distinct sites. The E (exit) site is the final position in this sequence. After the peptide bond is formed and the ribosome translocates, the uncharged, deacylated tRNA that was in the P-site is shifted into the E-site. From this position, the tRNA is subsequently released from the ribosome into the cytoplasm, where it can be recharged with another amino acid by its cognate synthetase.

**182.** (C) It is linked to the first mRNA nucleotide via an unusual 5'-to-5' triphosphate bridge.
The 5' cap of eukaryotic messenger RNA is characterized by a unique structural feature that distinguishes it from other nucleotides. It consists of a 7-methylguanosine residue attached to the first nucleotide of the transcript. Unlike standard phosphodiester bonds, which are 3'-to-

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**139.** (A) It hydrolyzes the ester bond of mispaired aminoacyl-tRNAs before they enter the ribosome.
Translational fidelity depends on the accurate attachment of amino acids to their cognate tRNAs by aminoacyl-tRNA synthetases. Many of these enzymes possess a specific editing domain that acts as a proofreading mechanism. If an incorrect amino acid is mistakenly attached to the tRNA, it is shifted into the editing site. The enzyme then hydrolyzes the ester bond, releasing the incorrect amino acid and the tRNA, thereby ensuring that only the correctly charged tRNA enters the ribosome.

**140.** (B) I, II, III, IV
Eukaryotic translation initiation proceeds through a highly coordinated series of steps. First, the 40S ribosomal subunit binds the initiator methionyl-tRNA to form the 43S pre-initiation complex. This complex is then recruited to the mRNA through the recognition of the 5' cap by the eIF4F complex. The 40S subunit then scans the mRNA in a 5'-to-3' direction to locate the start codon. Once found, the 60S large subunit joins to complete the functional 80S ribosome.

**141.** (D) Class I attaches the amino acid to the 2' OH; Class II to the 3' OH of the terminal adenosine.
Aminoacyl-tRNA synthetases are fundamentally divided into two classes based on their active site architecture and catalytic mechanisms. Class I enzymes utilize a Rossmann fold and typically catalyze the initial attachment of the amino acid to the 2' hydroxyl group of the terminal adenosine on the tRNA. In contrast, Class II enzymes feature a β-sheet fold and generally attach the amino acid directly to the 3' hydroxyl group. These differences represent two independent evolutionary solutions for tRNA aminoacylation.

**142.** (C) It base-pairs with the 16S rRNA to align the start codon in the P-site.
The Shine-Dalgarno sequence is a conserved purine-rich motif located in the 5' untranslated region of prokaryotic mRNA. It plays a critical role in translation initiation by base-pairing with a complementary pyrimidine-rich sequence at the 3' end of the 16S ribosomal RNA. This interaction physically tethers the 30S ribosomal subunit to the mRNA and precisely positions the AUG start codon into the ribosomal P-site, ensuring that translation begins at the correct location.

**143.** (B) II and III
Elongation factors are essential for the progression of translation. $eEF_1A$ is the eukaryotic functional homolog of the prokaryotic factor EF-Tu. Both factors are responsible for delivering aminoacyl-tRNAs to the ribosomal A-site. In bacteria, EF-Ts acts as a guanine nucleotide exchange factor (GEF) that regenerates active EF-Tu-GTP from the inactive EF-Tu-GDP complex. These mechanisms are highly conserved across species to maintain a steady supply of charged tRNAs for the synthesizing ribosome.

**144.** (D) It aligns tRNAs to facilitate peptidyl transfer via an induced-fit mechanism.
The 23S rRNA is the core catalytic component of the prokaryotic large ribosomal subunit. It functions as a ribozyme by providing the structural framework for the peptidyl transferase center. Rather than using conventional acid-base catalysis, the 23S rRNA works primarily through an induced-fit mechanism. It precisely orients the substrates in the A and P sites, lowering the activation energy and facilitating the spontaneous attack of the amino group on the peptidyl-tRNA ester bond.

**145.** (A) eIF4G
Translation termination in eukaryotes requires specific factors to recognize stop codons and release the completed polypeptide. $eRF_1$ identifies all three stop codons, and eRF3 is a GTPase that facilitates this process. The presence of a stop codon in the A-site is the trigger for termination. In contrast, eIF4G is an initiation factor involved in mRNA circularization and the recruitment of the small ribosomal subunit, playing no role in the termination phase of the protein synthesis cycle.

**146.** (C) Tetracycline binds the 30S subunit → Prevents aminoacyl-tRNA from entering the A-site → Halts elongation
Tetracycline is a broad-spectrum antibiotic that inhibits bacterial protein synthesis by specifically binding to the 30S ribosomal subunit. Its presence in the ribosomal A-site creates a physical blockage that prevents the entry of incoming aminoacyl-tRNA molecules. Because the tRNAs cannot bind to their respective codons, the process of elongation is effectively halted. This mechanism allows tetracycline to prevent the growth of a wide range of pathogenic

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bound, these proteins act as adapters that recruit histone deacetylases (HDACs). HDACs remove acetyl groups from histone tails, promoting the formation of compact, repressive heterochromatin. This structural shift physically occludes the transcriptional machinery, ensuring that the methylated gene remains inactive and inaccessible to RNA polymerase.

**190.** (C) I and II
RNA editing is a site-specific process that alters the nucleotide sequence of a transcript after it has been synthesized. In plant mitochondria and chloroplasts, the deamination of cytidine to uridine (C-to-U) is a common form of editing. In animals, adenosine-to-inosine (A-to-I) editing is widespread and is catalyzed by the ADAR enzyme family. These modifications occur post-transcriptionally throughout the transcript and are not restricted to the transcription start site, providing a flexible way to regulate protein function.

**191.** (B) The generation of mature siRNAs and miRNAs from their double-stranded precursors will be severely impaired.
Dicer is an essential ribonuclease III enzyme in the RNA interference pathway. Its primary function is to process long double-stranded RNAs and hairpin-shaped pre-microRNAs into small fragments approximately 21 to 23 nucleotides long. These small RNAs are the precursors to mature siRNAs and miRNAs. If a cell lacks functional Dicer, it cannot produce these small silencing molecules. Consequently, the entire post-transcriptional gene silencing machinery is crippled, leading to significant defects in gene regulation and viral defense.

**192.** (A) I, II, III, IV, V
Isolate a novel bioactive gene through metagenomics follows a logical experimental sequence. First, the environmental sample is collected, followed by the extraction of its total metagenomic DNA. This DNA is then used to construct a metagenomic library by cloning fragments into a host. The library is subjected to functional screening to identify clones with the desired enzymatic or biological activity. Finally, the specific gene responsible for that activity is isolated and characterized, enabling the discovery of unculturable genetic resources.

**193.** (A) Ferritin overexpression → Increased chelation and safe storage of iron in plant tissues → Higher bioavailable iron content
Biofortification for iron often targets the overexpression of ferritin, a universal iron-storage protein. Ferritin acts by sequestering iron atoms within its hollow spherical structure, protecting the cell from oxidative damage while maintaining a concentrated reservoir of the mineral. By increasing ferritin levels in the edible parts of a plant, such as rice grains, more iron is safely stored and concentrated. This results in a higher bioavailable iron content, making the crop a more effective source of nutrition for human populations.

**194.** (D) They are fundamentally limited because they act strictly as non-recyclable suicidal molecules.
Ribozymes are catalytic RNA molecules that offer several advantages in biotechnology and medicine. They can be engineered to specifically recognize and cleave target mRNA sequences, providing an alternative to other gene-silencing methods. Importantly, ribozymes function as true enzymes; after catalyzing a reaction, they are not consumed but are released to bind and cleave another target molecule. This ability to be recycled and perform multiple rounds of catalysis makes them powerful and efficient tools for the long-term downregulation of specific genes.

**195.** (A) Both A and R are true and R is the correct explanation of A.
Green Fluorescent Protein (GFP) is a unique and powerful biological tool because it does not require any external substrates or cofactors to fluoresce. Its fluorophore is formed entirely through an autocatalytic process within the core of its protein structure. This involves the spontaneous cyclization and oxidation of three specific internal amino acids: serine, tyrosine, and glycine. Because this reaction occurs naturally after the protein is translated, GFP can be used to track protein localization in virtually any living organism.

**196.** (D) Group I utilizes an external guanosine nucleophile; Group II uses an internal adenosine to form a lariat.
Group I and Group II introns are both self-splicing ribozymes, but they utilize distinct biochemical mechanisms. Group I introns initiate splicing using a free guanosine nucleotide as an external nucleophile, which attacks the 5' splice

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site. Group II introns use an internal nucleophile, specifically the 2' hydroxyl group of a conserved adenosine residue within the intron. This attack creates a characteristic lariat intermediate, a mechanism that is remarkably similar to the one used by the eukaryotic nuclear spliceosome.

**197.** (B) It adds adenine nucleotides to the 3' end of the mRNA strictly without utilizing a nucleic acid template.
Poly(A) polymerase is a specialized enzyme responsible for the 3' polyadenylation of eukaryotic messenger RNA. Unlike standard RNA polymerases, it does not read a DNA or RNA template to synthesize the poly-A tail. Instead, after the pre-mRNA is cleaved at a specific site, poly(A) polymerase adds a string of 100 to 250 adenine residues directly to the 3' end. This non-templated addition is essential for ensuring mRNA stability and promoting efficient translation by the ribosomal machinery.

**198.** (C) It may lack the complex regulatory switches or specific subcellular targeting signals provided by modifications.
Post-translational modifications, such as phosphorylation, glycosylation, and lipid attachment, add a critical layer of regulatory information to proteins. These modifications can act as molecular switches to control protein activity, influence folding and stability, and provide specific signals for trafficking to different organelles. A protein that lacks all such modifications may still be functional, but it would lack the sophisticated control mechanisms and precise targeting instructions that allow the cell to coordinate complex biological processes and respond to signals.

**199.** (D) To act as a specific recognition signal for the 26S proteasome to degrade the tagged protein
The covalent attachment of a chain of ubiquitin molecules, known as polyubiquitination, is a primary signal for protein turnover in eukaryotic cells. This ubiquitin chain acts as a highly specific "death tag" that is recognized by the regulatory subunit of the 26S proteasome. Once captured, the tagged protein is unfolded and fed into the proteasome's proteolytic core for degradation. This pathway is essential for the selective removal of misfolded, damaged, or no longer needed regulatory proteins to maintain cellular health.

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**200.** (A) I, II and IV
Translation initiation involves several specific factors across domains. In eukaryotes, eIF4E is the cap-binding protein that recognizes the 5' m7G cap of mRNA. eIF4A is an ATP-dependent RNA helicase that unwinds secondary structures in the 5' UTR to facilitate ribosomal scanning. In prokaryotes, IF-2 is a GTPase that directs the initiator tRNA to the P-site. Poly-A binding proteins, rather than eIF2, interact with the tail. Together, these factors ensure that the ribosome is correctly positioned for synthesis.

**201.** (B) Codon usage bias strongly correlates with the abundance of corresponding isoaccepting tRNAs in highly expressed genes.
Codon usage bias is the non-random selection of synonymous codons by an organism. This bias is not arbitrary; it is an evolutionary adaptation for translational efficiency. In genes that are expressed at high levels, there is a strong preference for "optimal" codons that match the most abundant tRNA species in the cell. This alignment minimizes the waiting time for the correct aminoacyl-tRNA to enter the ribosome, thereby maximizing the rate and accuracy of protein synthesis under conditions of high demand.

**202.** (B) It interacts with the large ribosomal subunit to facilitate proper tRNA docking.
The TΨC loop is one of the three major loops in the tRNA secondary structure and contains the modified base pseudouridine (Ψ). This loop plays a critical structural and functional role by interacting with the ribosomal RNA of the large subunit. This interaction is essential for the proper docking and orientation of the tRNA within the A and P sites of the ribosome. By stabilizing the tRNA-ribosome complex, the TΨC loop ensures that the translational machinery can accurately synthesize the polypeptide chain.

**203.** (A) It binds to the A-site post-peptidyl transfer, and its GTP hydrolysis drives a major conformational change that shifts the tRNAs into the P and E sites.
Translocation is a critical step in the elongation cycle where the ribosome moves along the mRNA by exactly one codon. This process is driven by Elongation Factor G (EF-G) in prokaryotes. EF-G binds to the ribosome in the A-site after peptide bond formation. The subsequent hydrolysis of GTP by EF-G triggers a massive

conformational change in the ribosome. This mechanical force "pushes" the mRNA and the associated tRNAs, shifting them into the P and E sites and clearing the A-site for the next tRNA.

**204.** (C) They serve as indispensable structural probes to dissect the distinct steps and functional centers of the ribosome.
Translational inhibitors like puromycin are more than just antibiotics; they are essential tools for molecular research. Because different inhibitors target specific steps or functional sites—such as decoding, peptidyl transfer, or translocation—they allow scientists to "freeze" the ribosome in various states. By observing the consequences of these blocks, researchers can map the intricate architecture of the ribosome and determine the precise function of its RNA and protein components. These chemical probes have been fundamental in our understanding of how proteins are synthesized.

**205.** (D) P-4, Q-2, R-1, S-3
Genetic regulation involves a combination of structural and functional elements. Cis-acting elements are DNA sequences on the same molecule that regulate adjacent genes. Trans-acting factors are diffusible proteins that bind to these sequences from elsewhere in the cell. Operons are clusters of related genes transcribed into a single polycistronic mRNA, typical in prokaryotes. Epigenetic marks are reversible modifications on DNA or histones that regulate gene accessibility. These components work together to provide a highly integrated system for controlling gene expression.

**206.** (C) It strictly initiates transcription entirely independent of a core promoter.
Enhancers are powerful regulatory elements that significantly increase the transcription of a gene. They are characterized by their ability to work over long distances, in either orientation, and from various positions. However, an enhancer cannot act alone; it functions by amplifying the activity of a promoter. It requires the presence of a core promoter to provide the actual site for the assembly of the general transcription factors and RNA polymerase. Therefore, enhancers modulate transcription but do not initiate it independently of the promoter complex.

**207.** (B) DNA Methyltransferase (DNMT) - Generally associated with transcriptional activation
Epigenetic modifications generally follow consistent patterns in gene regulation. Histone Acetyltransferases (HATs) add acetyl groups, promoting an open, active chromatin state. In contrast, DNA Methyltransferases (DNMTs) add methyl groups to cytosines, which is a classic signal for gene silencing and transcriptional repression. Histone Deacetylases (HDACs) remove acetyl groups, leading to chromatin condensation and repression. ATP-dependent remodeling complexes provide the mechanical force to move nucleosomes. Identifying DNA methylation as a signal for transcriptional activation is an incorrect characterization of this epigenetic process.

**208.** (D) The first transesterification step of splicing, forming the lariat, cannot occur.
The branch point sequence is a conserved internal motif within an intron that is essential for the splicing reaction. It contains a specific adenosine residue whose 2' hydroxyl group acts as the nucleophile for the first catalytic step. If this sequence is absent or non-functional, the branch point adenosine cannot attack the 5' splice site. Consequently, the characteristic lariat intermediate cannot be formed, and the entire splicing process is blocked. This highlights the critical role of the branch point in coordinating the removal of non-coding sequences.

**209.** (A) Differential inclusion of exons → Variable mRNA sequences → Production of diverse protein isoforms from one gene
Alternative splicing is a sophisticated regulatory mechanism that significantly increases the functional complexity of the proteome. During the processing of pre-mRNA, the spliceosome can choose different combinations of exons to be included in the final mature transcript. Each resulting mRNA sequence can then be translated into a different protein isoform. This allow a single gene to encode multiple proteins with distinct structural or functional properties, which can be tailored to the specific needs of different tissues or developmental stages.

**210.** (C) I and II
Small interfering RNAs (siRNAs) are a key component of the RNA interference pathway. They are produced when long double-stranded RNA precursors are cleaved by the enzyme Dicer.

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have evolved to be highly sensitive to specific environmental stresses, such as extreme drought or salt. In agricultural biotechnology, these promoters can be used to drive the expression of transgene defense genes. This ensures that the resistance trait is only turned "on" when the crop is actually under stress. By avoiding the metabolic cost of continuous, constitutive expression, this precisely regulated approach results in healthier crops and better yields under variable conditions.

**250.** (B) It enables physiological efficiency, allowing a cell to translate all 61 sense codons using fewer than 61 distinct tRNA species.

The wobble hypothesis is a fundamental principle of translation that provides a solution to the degeneracy of the genetic code. By allowing for non-standard base pairing at the third position of the codon, it significantly reduces the number of different tRNAs a cell needs to synthesize. A single tRNA can pair with several different codons that code for the same amino acid. This evolutionary adaptation increases the efficiency of the translational machinery, as the cell can successfully and accurately decode the entire genome while maintaining a smaller and more manageable pool of tRNA molecules.

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**217.** (B) The chaperone utilizes cycles of ATP binding and hydrolysis to alternately bind and release exposed hydrophobic segments, preventing premature aggregation.
Molecular chaperones facilitate protein folding through a dynamic process. They recognize and bind to hydrophobic regions of newly synthesized polypeptides that are not yet buried within a folded structure. This binding is regulated by ATP; the chaperone typically binds its substrate in one state and releases it after ATP hydrolysis. By cycling through these binding and release events, chaperones prevent the proteins from aggregating inappropriately with other molecules. This sequestration allows the protein multiple chances to fold into its correct, lowest-energy native conformation.

**218.** (B) A promoter precisely directs the initiation of transcription, whereas an enhancer amplifies the rate of transcription independent of precise distance or orientation.
Promoters and enhancers are distinct regulatory elements that work together to control gene expression. The promoter is located immediately upstream of a gene and serves as the essential landing site for RNA polymerase and general transcription factors to define the exact start of transcription. Enhancers are distal elements that can be located far from the gene. They act by binding specialized transcription factors that loop to the promoter, significantly increasing the frequency of transcription. While the promoter sets the "start," the enhancer controls the "volume" of gene expression.

**219.** (A) I, II and III
Eukaryotic cells utilize several coordinated pathways for protein degradation. Polyubiquitination targets intracellular proteins to the 26S proteasome, which uses ATP to unfold and digest them. Lysosomes are responsible for degrading extracellular proteins and cell-surface receptors through the processes of endocytosis and autophagy. Ubiquitin itself is a small, highly conserved regulatory protein, not a lipid. Together, these systems ensure the efficient removal of misfolded, damaged, or no longer required proteins, which is essential for maintaining cellular homeostasis and regulating signal transduction.

**220.** (A) I, II, III, IV
When tryptophan is abundant in the cell, the trp operon attenuation mechanism triggers premature termination of transcription. The process begins with RNA polymerase transcribing the leader sequence. Because tryptophan is plentiful, the ribosome follows closely and quickly translates the Trp codons in region 1. This ribosome position prevents region 2 from pairing with region 3. Consequently, region 3 base-pairs with region 4 to form a stable terminator hairpin, causing RNA polymerase to dissociate before it can reach the structural genes.

**221.** (D) It typically occurs on the cytosine residue within a CpG dinucleotide context.
DNA methylation is a fundamental epigenetic modification in eukaryotic organisms used for the long-term silencing of genes. In mammals and many other eukaryotes, this process involves the covalent attachment of a methyl group specifically to the carbon-5 position of the cytosine ring. This modification occurs predominantly where a cytosine is immediately followed by a guanine, a sequence known as a CpG dinucleotide. Clusters of these dinucleotides, called CpG islands, are often located in gene promoters where their methylation acts as a stable signal for transcriptional repression.

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**222.** (C) Because they possess intrinsic catalytic activity (ribozymes), they are considered remnants of the ancestral "RNA World."
Group I and Group II introns are self-splicing RNAs that function as ribozymes, catalyzing their own excision without the need for protein enzymes. This intrinsic catalytic ability is a profound evolutionary clue. It supports the "RNA World" hypothesis, which proposes that early in life's history, RNA molecules served both as the storage of genetic information and as the primary catalysts for biochemical reactions. These self-splicing introns are viewed as ancient molecular fossils that have persisted in modern genomes, reflecting the primordial functional versatility of RNA.

**223.** (B) It acts as a powerful transcriptional enhancer when bound to mRNA.
The RNA-Induced Silencing Complex (RISC) is the primary engine of post-transcriptional gene silencing. It is composed of a single-stranded guide RNA and several proteins, most notably a member of the Argonaute family. The complex

Once processed, these small double-stranded molecules are loaded into the RISC complex. One strand then acts as a guide to identify target mRNAs with high sequence complementarity. Once bound, the RISC complex cleaves the target mRNA, leading to its degradation. This sequence-specific mechanism is essential for regulating gene expression and defending the cell against exogenous viral RNAs.

**211.** (A) They may offer specific stress-inducible regulatory patterns adapted to extreme environmental conditions.
Plant bioprospecting in wild relatives is a valuable strategy for discovering novel regulatory elements. Wild species have evolved in natural, often harsh, environments and possess unique genetic adaptations. Their promoters may have evolved specialized motifs that trigger a rapid and robust gene expression response only when the plant is challenged by specific stresses like drought, salt, or extreme cold. Identifying and using these stress-inducible promoters in crop engineering allows for the development of resilient varieties that express defense traits only when they are needed.

**212.** (D) Enhancing the production of the phytosiderophore nicotianamine, which strongly chelates iron and facilitates its transport and storage in edible tissues.
Genetic biofortification for iron relies on understanding how plants move and store minerals. Nicotianamine is a natural chelator that binds to iron and zinc, forming stable complexes that can be transported through the plant's vascular system. By increasing the expression of the nicotianamine synthase gene, researchers can enhance the plant's ability to mobilize iron from the roots and deliver it to the seeds. This strategy effectively increases both the total concentration and the bioavailability of essential iron in the edible parts of staple crops like rice.

**213.** (B) To dramatically delay the evolution of resistance in target insect populations
Gene pyramiding for pest resistance involves incorporating several different genes, such as various Bt toxins, that target a pathogen through independent mechanisms. This "stacking" approach is designed to increase the durability of the resistance. For an insect population to overcome the crop's defenses, individuals would need to simultaneously evolve mutations to counter every single toxin. Because the probability of such a rare event is extremely low, gene pyramiding effectively slows down the development of resistance, extending the effective lifespan of the resistant crop variety in the field.

**214.** (C) It functions in vivo strictly to process the circular RNA genome of the virus during replication.
The Hepatitis Δ Virus (HDV) ribozyme is a small, specialized RNA molecule that is an integral part of the viral replication cycle. During replication, the virus produces long, multimeric RNA transcripts through a rolling-circle mechanism. The HDV ribozyme catalyzes a site-specific self-cleavage reaction that breaks these long strands into individual, genome-sized units. This ribozyme is unique because it is highly stable and active within the environment of the human cell nucleus, providing an essential enzymatic function for the propagation of the viral RNA genome.

**215.** (A) Both A and R are true and R is the correct explanation of A.
The genetic code is one of the most fundamental and universally conserved aspects of biology. It defines the mapping between the 64 possible codons and the 20 standard amino acids used to build proteins. While minor variations exist, particularly in mitochondria and some specialized microorganisms, the same standard code is utilized by the vast majority of prokaryotic and eukaryotic nuclear genomes. This near-universal conservation suggests that the code was established very early in evolution and has remained remarkably stable because any major change would likely be lethal.

**216.** (A) Spatial flexibility at the 5' position of the anticodon (which pairs with the 3' position of the codon)
The wobble hypothesis, proposed by Francis Crick, explains how the cell can translate 61 codons using fewer than 61 tRNAs. It suggests that the standard Watson-Crick base-pairing rules are strictly enforced only for the first two positions of the codon. The third position of the codon (pairing with the first base, or 5' position, of the anticodon) allows for spatial flexibility or "wobble." This relaxed pairing allows a single tRNA to recognize several synonymous codons, provided they differ only at the third position.

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**230.** (A) II and III
Epigenetic modifications are changes that affect gene activity without altering the underlying DNA sequence. These marks, such as DNA methylation and histone acetylation, are critical for cellular differentiation, as they allow different cell types to express different genes from the same genome. Importantly, many of these marks are potentially heritable, meaning they can be passed on through cell division. Epigenetics provides a mechanism for the cell to integrate environmental signals and maintain stable gene expression patterns throughout the life of the organism.

**231.** (D) It often contains regulatory sequences that influence mRNA stability, localization, and translational efficiency.
The 3' untranslated region (UTR) is the section of mRNA located between the stop codon and the poly-A tail. While it does not code for amino acids, it is far from "junk" sequence. The 3' UTR contains numerous regulatory elements, such as binding sites for microRNAs and various RNA-binding proteins. These interactions control how long the mRNA survives in the cell, where it is located within the cytoplasm, and how efficiently it is translated by the ribosome, providing a powerful layer of post-transcriptional control.

**232.** (A) The systematic assembly of multiple desirable genes, typically for resistance or quality traits, into a single elite genotype
Gene pyramiding is a sophisticated breeding and biotechnological strategy aimed at combining several different genes into a single plant variety. Each gene typically provides a different mechanism for a specific trait, such as resistance to a particular pest or improved nutritional quality. By "stacking" these genes, breeders can create crops that are more resilient to evolving pathogens and have more robust desirable characteristics. This approach is much more effective and durable than relying on a single gene, which is more likely to be overcome by environmental challenges or pathogen evolution.

**233.** (B) To allow for the gentle, site-specific removal of the tag after purification, yielding native target protein
Affinity tags are invaluable for the purification of recombinant proteins, but their presence can sometimes interfere with the protein's final structure or biological activity. To address this, researchers often engineer a specific protease cleavage site between the tag and the target protein. After the fusion protein is purified on an affinity column, it is treated with a specialized protease that recognizes only that site. This allows for the precise removal of the tag under mild conditions, leaving behind the purified, native protein for functional studies or therapeutic use.

**234.** (B) They guide the RISC complex to bind imperfectly to the 3' UTR of target mRNAs, heavily impeding ribosomal progression or initiation.
MicroRNAs (miRNAs) are key regulators of gene expression that typically work through translational repression. Unlike siRNAs, which often match their targets perfectly for cleavage, miRNAs usually bind with partial complementarity to sites in the 3' untranslated region of their target mRNAs. This imperfect binding by the RISC complex does not necessarily lead to immediate cleavage of the transcript. Instead, it physically and biochemically interferes with the translational machinery, preventing the ribosome from initiating translation or moving along the mRNA, thus reducing the production of the target protein.

**235.** (A) Both A and R are true and R is the correct explanation of A.
Genetic biofortification is a sustainable, long-term strategy for addressing micronutrient deficiencies in developing regions. Unlike supplementation programs, which require expensive, continuous distribution of vitamins or minerals, biofortified crops provide the nutrients directly through the food supply. Once the seeds are introduced, they can be saved and replanted by local farmers. This allows the improved nutritional traits to become part of the local agricultural system, ensuring that subsequent generations have access to essential nutrients without the need for external intervention.

**236.** (C) The highly precise removal of non-coding introns and the precise ligation of coding exons
RNA splicing is a fundamental step in the processing of eukaryotic pre-mRNA into mature mRNA. Most eukaryotic genes are composed of coding regions called exons separated by non-coding regions called introns. Splicing involves the recognition of specific sequences at the intron-exon boundaries. The spliceosome, a

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complex machine made of RNA and proteins, then performs two chemical reactions to cut out the introns and join the exons together in the correct order. This precision is essential because even a single nucleotide error could disrupt the reading frame.

**237.** (C) A single regulatory promoter controlling the transcription of multiple contiguous structural genes into one mRNA
An operon is a fundamental unit of genetic organization in prokaryotes. It consists of a cluster of structural genes that usually encode proteins involved in the same metabolic pathway. These genes are located next to each other on the chromosome and are controlled by a single promoter. Consequently, they are transcribed together into a single, long piece of mRNA called a polycistronic transcript. This arrangement allows the bacterium to coordinate the expression of all the proteins needed for a specific task in response to a single environmental signal.

**238.** (C) It utilizes identical repressor mechanisms to the prokaryotic lac operon.
Eukaryotic gene regulation is significantly more complex than the relatively simple repressor-operator systems found in prokaryotes. In eukaryotes, transcription involves the coordinated action of many distal enhancers, core promoters, and large coactivator complexes like Mediator. It also requires the dynamic remodeling of chromatin structure to make DNA accessible. While prokaryotes often use a single repressor to block an operon, eukaryotes use a sophisticated network of multiple activators and repressors that integrate various signals through DNA looping and protein-protein interactions to fine-tune gene expression.

**239.** (B) Tracking the rapid, real-time intracellular movement of a protein within a live, growing root
The GUS reporter system is a classic tool for studying gene expression in plants, but it has specific limitations. The assay involves applying a substrate (X-Gluc) that is cleaved to form a blue precipitate, a process that typically requires the tissue to be killed and fixed. Therefore, while GUS is excellent for mapping the tissue-specific and developmental patterns of a promoter, it cannot be used to observe the real-time dynamics or movement of proteins in living cells. For such dynamic, live-cell studies, fluorescent reporters like GFP are much more appropriate.

**240.** (D) I, II and IV
The hammerhead ribozyme is a small catalytic RNA characterized by its distinct structural motifs. It is composed of three base-paired helices that surround a highly conserved catalytic core. To achieve its full catalytic potential, the ribozyme requires a divalent metal ion, such as magnesium, which stabilizes its three-dimensional fold and participates in the chemical reaction. Importantly, this ribozyme is truly autocatalytic; it does not require any protein enzymes to function. These features make it a powerful and efficient tool for site-specific RNA cleavage in molecular biology and biotechnology.

**241.** (A) dsRNA formation → Dicer processing → siRNA/RISC complex → Target mRNA recognition → mRNA cleavage
RNA-directed gene silencing follows a highly organized biological pathway. It begins with the presence or formation of long double-stranded RNA (dsRNA). The enzyme Dicer recognizes and processes this dsRNA into small interfering RNAs (siRNAs). These fragments are then loaded into the RISC complex. The complex uses one strand of the siRNA as a guide to locate a complementary target mRNA. Once the target is recognized, the catalytic component of the RISC complex cleaves the mRNA, leading to its degradation and the successful silencing of the target gene.

**242.** (C) It is significantly less likely to experience a rapid breakdown in resistance compared to a monoculture expressing a single resistance trait.
Gene pyramiding provides a major evolutionary advantage for crop protection. In a field where plants express only one resistance gene, any individual pest with a single mutation that overcomes that defense can quickly multiply. However, in a pyramided crop with multiple stacked resistance genes, a pest would need several specific, simultaneous mutations to survive. Because the likelihood of such multiple mutations occurring in one individual is extremely low, the resistance in the crop is much more stable and "durable," effectively preventing the rapid breakdown often seen with single-gene traits.

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**243.** (A) Codon-anticodon recognition - 40S subunit decoding center (A-site)
During the elongation phase of translation, the accurate selection of the correct tRNA is essential. This critical "decoding" step occurs in the small ribosomal subunit (30S in prokaryotes, 40S in eukaryotes). As each incoming aminoacyl-tRNA enters the ribosomal A-site, its anticodon is checked against the mRNA codon. Only when there is a correct match does the ribosome undergo a conformational change that allows the tRNA to be fully accommodated. This decoding center ensures that the genetic information is accurately translated into the correct sequence of amino acids.

**244.** (D) The His-tag is smaller (typically 6-10 amino acids) and is much less likely to interfere with the target protein's structure or function.
One of the primary advantages of the His-tag over larger fusion partners like GST is its minimal size. Consisting of only a few histidine residues, the His-tag is less likely to significantly alter the folding, solubility, or biological activity of the target protein. This often means that the tag does not need to be removed for downstream applications. In contrast, larger tags like GST (approximately 26 kDa) can sometimes cause steric hindrance or lead to protein aggregation, frequently requiring an extra step of protease cleavage after the purification process is complete.

**245.** (B) It binds to the 30S A-site to prevent premature entry of the initiator tRNA, forcing it into the P-site.
Initiation Factor 1 (IF-1) is a small but essential protein in prokaryotic translation. Its primary function is to bind to the A-site of the 30S ribosomal subunit during the assembly of the initiation complex. This binding physically blocks the A-site, ensuring that the initiator fMet-tRNAfMet can only enter the P-site where it must pair with the start codon. By directing the initiator tRNA to the correct location and preventing the entry of other aminoacyl-tRNAs, IF-1 ensures the high fidelity and accuracy of the translation initiation process.

**246.** (C) It relies predominantly on the specific spacing between the -3 and +4 positions relative to the AUG start codon.
The Kozak consensus sequence is a key regulatory motif that determines the efficiency of translation initiation in eukaryotic cells. It is not a rigid sequence but rather a set of preferences for specific nucleotides surrounding the AUG start codon. The most influential positions are the purine (usually A) at the -3 position and the guanine at the +4 position relative to the start site. By providing the optimal context, this consensus helps the ribosome to correctly identify the true start codon during the scanning process, ensuring efficient and accurate protein production.

**247.** (D) Kinase activity → Addition of bulky negatively charged phosphate group → Conformational change → Altered protein activity
Protein phosphorylation is a universal molecular switch that regulates cellular activity. Enzymes called kinases catalyze the addition of a phosphate group to specific serine, threonine, or tyrosine residues. The introduction of this large, negatively charged group can cause a significant shift in the protein's internal electrostatic interactions, leading to a change in its three-dimensional shape. This conformational change can activate or deactivate the protein, alter its ability to interact with other molecules, or change its location within the cell, providing a rapid and reversible way to control biological pathways.

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**248.** (A) It specifically cleaves the N-terminal signal sequence from the growing polypeptide chain after it enters the ER lumen.
Signal peptidase is an essential enzyme located in the membrane of the endoplasmic reticulum. Its role is to process proteins that are being translocated into the ER. As the hydrophobic signal peptide enters the ER lumen, signal peptidase recognizes a specific cleavage site and removes the signal sequence from the rest of the polypeptide chain. This maturation step is critical for the proper folding and further trafficking of secretory and membrane proteins. Once the signal is removed, the mature protein is released into the ER lumen for subsequent modification.

**249.** (B) Developing precisely regulated transgenic crops that express stress-resistance traits only when challenged by that specific stress.
The discovery of novel promoters from wild plants is a major goal of plant bioprospecting. Wild relatives often possess promoters that

uses the guide RNA to scan and identify complementary mRNA targets. Once bound, RISC functions to silence the gene, either by cleaving the target mRNA (slicer activity) or by physically blocking the translational machinery. Its role is strictly inhibitory; it does not enhance transcription or the production of the target protein.

**224.** (D) Environmental samples collected from polar ice caps or deep-ocean thermal vents
Metagenomic bioprospecting for industrial enzymes often targets organisms living in extreme environments. To find "cold-active" enzymes, researchers look for extremophiles, known as psychrophiles, that have evolved to thrive at very low temperatures. These organisms are typically found in samples from polar regions or deep-sea environments. The enzymes they produce have evolved to maintain catalytic efficiency in the cold, making them ideal for industrial applications such as cold-water detergents or food processing, where standard enzymes would be inactive.

**225.** (A) P-3, Q-4, R-2, S-1
Translation involves specific sequences and factors that are often unique to either prokaryotes or eukaryotes. The Kozak sequence is a eukaryotic motif that optimizes start codon recognition, while the Shine-Dalgarno sequence is its prokaryotic counterpart. $eEF_1A$ is the eukaryotic elongation factor responsible for tRNA delivery to the ribosome. Hsp60 is a chaperonin that provides an isolated chamber to facilitate proper protein folding. These components represent the specialized machinery that ensures the accuracy and efficiency of protein synthesis and maturation across different cellular domains.

**226.** (C) The polyhistidine sequence strongly coordinates transition metal ions → The fusion protein specifically binds to IMAC columns
The His-tag is a widely used affinity tag because of the unique chemical properties of the histidine side chain. A string of six to ten histidines can form strong coordination bonds with divalent transition metal ions like nickel or cobalt. In protein purification, these ions are immobilized on a chromatographic resin, a technique called Immobilized Metal Affinity Chromatography (IMAC). When a cell lysate is passed over the column, only the His-tagged fusion protein is captured. This allows for a very efficient, one-step purification of the recombinant protein from a complex mixture.

**227.** (A) They are highly susceptible to degradation by ubiquitous cellular and extracellular ribonucleases.
While ribozymes have great potential as therapeutic agents due to their ability to specifically target and cleave disease-causing RNAs, they face a significant biological hurdle. RNA is inherently much less stable than DNA or proteins. In the body, ribozymes are rapidly broken down by ribonucleases (RNases), which are found in the blood and inside every cell. To make ribozyme therapy effective, researchers must develop chemical modifications or specialized delivery systems to protect these catalytic RNAs from degradation and ensure they reach their targets in a functional state.

**228.** (D) The start codon sets the exact reading frame for the entire subsequent translation process.
Translation is a process of decoding mRNA in non-overlapping groups of three nucleotides. The identification of the AUG start codon is the most critical step in initiation because it establishes the reading frame. From that point on, the ribosome reads each subsequent triplet as a codon for a specific amino acid. If the ribosome starts even one nucleotide off, the entire downstream sequence will be misinterpreted, leading to a completely different and usually non-functional protein. This is why the precision of start codon recognition is essential.

**229.** (B) SRP - Cytoplasmic ribonucleoprotein that recognizes the signal peptide
The secretory pathway relies on the Signal Recognition Particle (SRP) to ensure that specific proteins are correctly targeted to the endoplasmic reticulum. As the signal peptide emerges from the ribosome, the SRP binds to it, pausing translation. The entire complex then docks with the SRP receptor on the ER membrane. Other components include the Sec61 translocon, which forms the pore for entry, and signal peptidase, which removes the signal sequence inside the ER. This highly coordinated system ensures that secretory and membrane proteins are properly localized during their synthesis.

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# CHAPTER 09
# Plant Molecular Biology

2016

**1.** Which phytochrome domain is primarily responsible for the covalent attachment of the phytochromobilin chromophore?
(A) GAF domain
(B) PAS domain
(C) PHY domain
(D) PRD domain

2010

**2.** What specific amino acid residue in the apophytochrome covalently links to the linear tetrapyrrole chromophore?
(A) Tyrosine
(B) Serine
(C) Cysteine
(D) Histidine

2023

**3.** During the Pr to Pfr conversion, what major structural change occurs in the chromophore?
(A) Cis to trans isomerization at the C15 double bond
(B) Trans to cis isomerization at the C15 double bond
(C) Oxidation of the central pyrrole ring
(D) Reduction of the D-ring propionate side chain

2023

**4.** Which of the following wavelengths of light most effectively converts the inactive Pr form to the active Pfr form?
(A) 450 nm
(B) 660 nm
(C) 730 nm
(D) 800 nm

2004

**5.** Following photoconversion to the Pfr state, what is the primary subcellular destination of phytochrome A (phyA)?
(A) Chloroplast stroma
(B) Cell nucleus
(C) Mitochondrial matrix
(D) Endoplasmic reticulum

2001

**6.** Which plant specific transport proteins are specifically required for the nuclear import of phytochrome A under far-red light?
(A) PIF3 and PIF4
(B) COP1 and SPA1
(C) FHY1 and FHL
(D) Importin $\alpha$ and $\beta$

2012

**7.** What is the molecular consequence of phytochrome interaction with Phytochrome Interacting Factors (PIFs) in the nucleus?
(A) Activation of PIF transcriptional activity
(B) Export of PIFs back to the cytoplasm
(C) Stabilization of the PIF-DNA binding complex
(D) Rapid phosphorylation and subsequent degradation of PIFs

2011

**8.** Which statement INCORRECTLY describes the physiological role of the Pfr form of phytochrome?
(A) It promotes hypocotyl elongation in dark-grown seedlings
(B) It initiates seed germination in light-sensitive seeds
(C) It stimulates leaf expansion
(D) It regulates flowering time in photoperiodic plants

2010

**9.** By what mechanism does the active phytochrome molecule directly alter gene expression?
(A) Acting as a histone acetyltransferase
(B) Interacting with and regulating transcriptional regulators
(C) Binding directly to TATA boxes
(D) Cleaving specific mRNA transcripts

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2014

**10.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Phytochrome B (phyB) is primarily localized in the cytoplasm in dark-grown seedlings.
Reason (R): The nuclear localization signal of

2009

**30.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): RuBisCO activase is strictly required to maintain RuBisCO in an active state during steady-state photosynthesis.
Reason (R): RuBisCO activase directly provides the $CO_2$ molecule to the active site of RuBisCO.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**31.** Which post-translational modification is required on the epsilon-amino group of a specific lysine residue for RuBisCO activation?
(A) Phosphorylation
(B) Carbamylation
(C) Ubiquitination
(D) Methylation

2001

**32.** What metal ion coordinates with the carbamylated lysine to stabilize the active site of RuBisCO?
(A) Iron ($Fe^{2+}$)
(B) Copper ($Cu^{2+}$)
(C) Zinc ($Zn^{2+}$)
(D) Magnesium ($Mg^{2+}$)

2023

**33.** Which enzyme catalyzes the primary carbon fixation step in the mesophyll cells of C4 plants?
(A) Phosphoenolpyruvate carboxylase (PEPC)
(B) RuBisCO
(C) Pyruvate orthophosphate dikinase (PPDK)
(D) NADP-malic enzyme

2003

**34.** What is the substrate for Phosphoenolpyruvate carboxylase (PEPC) in C4 photosynthesis?
(A) $CO_2$ and RuBP
(B) $CO_2$ and Phosphoenolpyruvate
(C) $HCO_3^-$ and Phosphoenolpyruvate
(D) $HCO_3^-$ and Pyruvate

2023

**35.** Consider the following statements concerning the regulation of PEPC in C4 plants:
I. PEPC is activated by malate.
II. PEPC is allosterically activated by glucose-6-phosphate.
III. PEPC kinase activity increases in response to light.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) II

2022

**36.** How does phosphorylation affect the kinetic properties of C4 PEPC?
(A) It decreases its affinity for PEP
(B) It increases its sensitivity to malate inhibition
(C) It targets the enzyme for degradation
(D) It decreases its sensitivity to malate inhibition

2012

**37.** Which statement describing PEPC kinase is most accurate?
(A) It is regulated primarily by calcium/calmodulin binding
(B) Its activity is primarily controlled by strictly de novo protein synthesis and degradation
(C) It is a highly stable protein that remains active in the dark
(D) It relies on cAMP as a second messenger

2003

**38.** In which specific cellular compartment is C4 PEPC localized?
(A) Mesophyll chloroplast stroma
(B) Bundle sheath chloroplast stroma
(C) Mesophyll cytosol
(D) Bundle sheath cytosol

2005

**39.** What is the physiological consequence of daytime PEPC phosphorylation in C4 plants?
(A) It halts carbon fixation to prevent excessive malate accumulation
(B) It shifts the enzyme specificity from $HCO_3^-$ to $CO_2$
(C) It promotes the immediate export of PEPC to the bundle sheath
(D) It ensures PEPC remains highly active despite rising cytosolic malate levels

2022

**40.** Match the photorespiratory enzyme with its primary organelle of localization.
List I: (Enzyme)
P. Glycolate oxidase
Q. Serine hydroxymethyltransferase
R. Phosphoglycolate phosphatase
S. Glycerate kinase

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Q. GUN4
R. GUN5
S. PTM

List II: (Function)

1. Chloroplast envelope bound transcription factor
2. Central integrator of multiple retrograde signals
3. Porphyrin-binding protein in tetrapyrrole synthesis
4. ChlH subunit of Mg-chelatase

(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-1, R-2, S-3

2022

**21.** Which molecule serves as a widely accepted tetrapyrrole intermediate acting as a retrograde signal from the chloroplast?
(A) Protoporphyrin IX
(B) Mg-protoporphyrin IX
(C) Protochlorophyllide
(D) Phytochromobilin

2003

**22.** What is the ultimate effect of chloroplast-to-nucleus retrograde signaling under severe photooxidative stress?
(A) Upregulation of chlorophyll biosynthesis genes
(B) Inhibition of mitochondrial respiration
(C) Downregulation of photosynthesis-associated nuclear genes (PhANGs)
(D) Immediate cell division and proliferation

2022

**23.** Which chloroplast-localized protein undergoes proteolytic cleavage to release a cytosolic fragment that enters the nucleus during retrograde signaling?
(A) GUN1
(B) PRIN2
(C) ABI4
(D) PTM (PHD type transcription factor with transmembrane domain)

2017

**24.** What nuclear transcription factor frequently acts downstream of chloroplast retrograde signals to repress PhANGs?
(A) ABI4
(B) HY5
(C) PIF3
(D) FHY1

2023

**25.** Consider the following statements regarding RuBisCO gene expression:

I. The large subunit (rbcL) is encoded by the nuclear genome.
II. The small subunit (rbcS) is encoded by the chloroplast genome.
III. rbcS transcripts are translated on cytosolic 80S ribosomes.
IV. Assembly of the holoenzyme occurs in the chloroplast stroma.

Which of the above statements are INCORRECT?
(A) I and II
(B) III and IV
(C) I, II and IV
(D) I, III and IV

2021

**26.** How is the small subunit of RuBisCO (RBCS) transported into the chloroplast?
(A) Via vesicle trafficking from the Golgi
(B) Via an N-terminal transit peptide recognized by TOC/TIC complexes
(C) Through simple diffusion across the envelope
(D) As a co-translational mechanism using chloroplast ribosomes

2011

**27.** What is the quaternary structure of the functional RuBisCO holoenzyme in higher plants?
(A) Eight large and eight small subunits (L8S8)
(B) Four large subunits only (L4)
(C) Two large and two small subunits (L2S2)
(D) A single large polypeptide chain

2005

**28.** Which essential chaperone complex facilitates the correct folding of the newly synthesized RuBisCO large subunit in the stroma?
(A) HSP90
(B) Trigger Factor
(C) GroEL/GroES-like chaperonin complex (Cpn60/Cpn10)
(D) Calnexin

2009

**29.** What specific ancillary protein is required for the proper assembly of RuBisCO large and small subunits into the L8S8 holoenzyme?
(A) RuBisCO activase
(B) GUN1
(C) PIF4
(D) $RAF_1$ (RuBisCO Accumulation Factor 1)

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2013

**92.** Which amino acid is exceptionally abundant in metallothioneins and provides the sulfhydryl groups necessary for heavy metal chelation?
(A) Methionine
(B) Histidine
(C) Cysteine
(D) Proline

2014

**93.** What serves as the direct metabolic precursor for the enzymatic synthesis of phytochelatins by Phytochelatin Synthase (PCS)?
(A) Glutamine
(B) S-adenosylmethionine
(C) Cysteine alone
(D) Glutathione (GSH)

2003

**94.** How is Phytochelatin Synthase (PCS) regulated in the plant cell?
(A) By heavy metal-induced transcriptional upregulation only
(B) By phosphorylation via MAPK cascades
(C) Through direct post-translational activation by heavy metal ions binding to the enzyme
(D) By targeted proteolysis in the absence of metals

2017

**95.** Following the formation of heavy metal-phytochelatin complexes in the cytosol, where are they primarily transported for long-term sequestration?
(A) Chloroplast
(B) Central vacuole
(C) Apoplast
(D) Endoplasmic reticulum

2023

**96.** Which family of tonoplast-localized transporters typically mediates the vacuolar compartmentalization of heavy metal-phytochelatin complexes?
(A) NRAMP family transporters
(B) ZIP family transporters
(C) P-type ATPases
(D) ABCC-type ABC transporters

2001

**97.** Which transition metal, essential at low concentrations but toxic at high levels, is primarily loaded into the vacuole by the MTP1 transporter?
(A) Zinc
(B) Cadmium
(C) Lead
(D) Arsenic

2025

**98.** In response to sudden elevated temperatures, how are Heat Shock Factors (HSFs) activated in the cytoplasm?
(A) They are synthesized purely de novo
(B) They are demethylated at specific lysine residues
(C) They are released from repressing complexes containing HSP70/HSP90
(D) They are targeted for SUMOylation and degradation

2014

**99.** Upon activation, what structural configuration do Heat Shock Factors (HSFs) adopt to bind DNA efficiently?
(A) Trimers
(B) Monomers
(C) Dimers
(D) Tetramers

2022

**100.** Match the Heat Shock Protein (HSP) family with its primary characteristic function.
List I: (HSP Family)
P. sHSP (small HSPs)
Q. HSP70
R. HSP90
S. HSP100
List II: (Function)
1. ATP-dependent unfolding and disaggregation of severely clumped proteins
2. Co-chaperone dependent refolding of misfolded proteins
3. Late-stage folding and regulation of client signaling proteins (e.g., receptors)
4. ATP-independent binding to denatured proteins to prevent initial aggregation
(A) P-4, Q-2, R-3, S-1
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-2, Q-4, R-1, S-3

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2006

**101.** Which cis-acting regulatory element is bound by trimeric HSFs in the promoters of heat shock responsive genes?
(A) HSE (Heat shock element)
(B) DRE (Dehydration-responsive element)
(C) ABRE (ABA-responsive element)
(D) LRE (Light-responsive element)

List II: (Organelle)
1. Chloroplast
2. Peroxisome
3. Mitochondrion
4. Cytosol

(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-3, R-1, S-1
(C) P-3, Q-1, R-2, S-2
(D) P-2, Q-3, R-4, S-1

2016

**41.** What is the initial toxic product generated by the oxygenase activity of RuBisCO?
(A) 3-Phosphoglycerate
(B) 2-Phosphoglycolate
(C) Glyoxylate
(D) Hydrogen peroxide

2025

**42.** During photorespiration, in which organelle does the conversion of glycine to serine occur?
(A) Chloroplast
(B) Mitochondrion
(C) Peroxisome
(D) Vacuole

2010

**43.** The mitochondrial glycine decarboxylase complex (GDC) comprises four specific subunits. Which of the following is NOT one of them?
(A) M-protein (magnesium binding)
(B) P-protein (pyridoxal phosphate dependent)
(C) H-protein (lipoamide containing)
(D) T-protein (tetrahydrofolate dependent)

2023

**44.** What critical molecule is released into the mitochondrial matrix during the GDC-catalyzed reaction in photorespiration?
(A) Oxygen
(B) Urea
(C) Ammonia
(D) Nitric oxide

2021

**45.** Consider the following statements regarding the photorespiratory (C2) cycle:
I. It consumes ATP and reducing equivalents.
II. It completely recovers all carbon atoms lost to oxygenation.
III. It prevents photoinhibition under high light and low $CO_2$.
IV. It involves cross-talk between three distinct organelles.
Which of the above statement(s) are INCORRECT?
(A) I and III
(B) II
(C) I, III and IV
(D) II and IV

2004

**46.** How does the plant cell reassimilate the massive amounts of ammonia released during photorespiration?
(A) Through the Glutamate Dehydrogenase (GDH) pathway in mitochondria
(B) By directly excreting it through the stomata
(C) Through the GS/GOGAT cycle in the chloroplast
(D) By converting it to nitrate in the peroxisome

2025

**47.** Which enzyme is responsible for converting hydroxypyruvate to glycerate in the peroxisome?
(A) Glycolate oxidase
(B) Hydroxypyruvate reductase
(C) Serine:glyoxylate aminotransferase
(D) Glycerate kinase

2014

**48.** The nitrogenase enzyme complex consists of two major components. What are they?
(A) Ferredoxin and Flavodoxin
(B) NifA and NifL
(C) Nod factor and Symbiosome
(D) Fe-protein and MoFe-protein

2007

**49.** What is the primary role of the Fe-protein in the nitrogenase complex?
(A) It acts as an ATP-dependent reductase, transferring electrons to the MoFe-protein
(B) It binds molecular nitrogen directly
(C) It synthesizes the iron-molybdenum cofactor
(D) It protects the complex from oxygen damage

2007

**50.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The nitrogenase complex is rapidly and irreversibly inactivated by molecular oxygen.
Reason (R): Molecular oxygen acts as a competitive inhibitor by binding precisely to the FeMoco active site, blocking nitrogen binding.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.

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(D) A is false but R is true.

2001

**51.** Where is the active site for the reduction of N2 to NH3 located within the nitrogenase complex?
(A) In the FeMoco (Iron-Molybdenum cofactor) of the MoFe-protein
(B) In the [4Fe-4S] cluster of the Fe-protein
(C) In the P-cluster of the MoFe-protein
(D) In the interface between the Fe-protein and ATP

2006

**52.** How many molecules of ATP are minimally hydrolyzed by nitrogenase for the reduction of one molecule of N2 to two molecules of NH3?
(A) 4
(B) 8
(C) 16
(D) 32

2014

**53.** Which gene products are primarily responsible for the biosynthesis and assembly of the FeMoco in diazotrophs?
(A) nifH, nifD, and nifK
(B) nifB, nifE, nifN, and nifV
(C) nifA and nifL
(D) nodA, nodB, and nodC

2003

**54.** What unusual molecule is centrally located within the core of the FeMoco cluster?
(A) A sulfur atom
(B) A nitrogen atom
(C) A carbon atom
(D) A magnesium atom

2008

**55.** Consider the following statements regarding protection against oxygen in nitrogen-fixing systems:
I. Leghemoglobin buffers free oxygen in legume nodules.
II. Azotobacter employs extremely high respiration rates to scavenge oxygen.
III. Cyanobacteria use heterocysts lacking Photosystem I to avoid oxygen.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I

2003

**56.** Which regulatory protein acts as the master transcriptional activator for the expression of nif operons in Klebsiella pneumoniae?
(A) NifA
(B) NifL
(C) NtrC
(D) FixL

2009

**57.** Under what specific environmental conditions does NifL inhibit the activity of NifA?
(A) Low oxygen and low combined nitrogen
(B) High oxygen and high combined nitrogen
(C) High temperature and low pH
(D) High ATP/ADP ratios

2007

**58.** How does NtrC regulate nitrogen fixation at the molecular level?
(A) It directly represses all nif structural genes
(B) It activates the transcription of the nifLA operon under nitrogen-limiting conditions
(C) It acts as a protease that degrades NifA
(D) It binds oxygen to prevent nitrogenase destruction

2024

**59.** In the symbiotic Rhizobium system, the FixL-FixJ two-component regulatory system controls nitrogen fixation genes. What is FixL?
(A) A heme-based histidine kinase that acts as an oxygen sensor
(B) A DNA-binding response regulator
(C) A transport protein for molybdenum
(D) An enzyme that synthesizes Nod factors

2009

**60.** Match the symbiotic regulatory component with its primary function.
List I: (Component)
P. FixL
Q. FixJ
R. NifA
S. RpoN (Sigma 54)
List II: (Function)
1. Transcriptional activator of nif and fix structural genes
2. Sensor kinase detecting oxygen levels
3. Alternative sigma factor required for NifA-dependent transcription
4. Response regulator activated by phosphorylation
(A) P-1, Q-4, R-2, S-3
(B) P-2, Q-4, R-1, S-3
(C) P-4, Q-2, R-3, S-1

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(D) P-3, Q-1, R-4, S-2

2008

**61.** Which type of bacterial promoter sequence is recognized by the RpoN (Sigma 54) holoenzyme for nif gene transcription?
(A) -10 and -35 sequences
(B) -12 and -24 sequences
(C) TATA box
(D) CCAAT box

2014

**62.** What structural feature of NifA allows it to interact with the RNA polymerase-Sigma 54 complex from a distance?
(A) A transmembrane helix
(B) A leucine zipper
(C) An enhancer-binding AAA+ ATPase domain
(D) A zinc finger motif

2017

**63.** During the initial molecular dialogue of Rhizobium-legume symbiosis, what plant-derived compounds act as the primary chemoattractants and signals?
(A) Gibberellins
(B) Flavonoids and isoflavonoids
(C) Strigolactones
(D) Jasmonic acid

2003

**64.** What bacterial protein binds the plant-derived flavonoids and acts as a transcriptional activator for nodulation (nod) genes?
(A) NodA
(B) NodC
(C) SymRK
(D) NodD

2012

**65.** Consider the following statements regarding the core structure of Nod factors:
I. They are lipo-chitooligosaccharides.
II. They consist of a β-1,4-linked N-acetyl-D-glucosamine backbone.
III. They always contain a specific pentose sugar at the reducing end.
IV. They feature an N-linked fatty acyl chain at the non-reducing end.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) II and IV
(C) I, III and IV
(D) I and IV

2021

**66.** Which specific plant receptor-like kinases recognize the bacterial Nod factors to initiate the symbiotic signaling cascade?
(A) FLS2 and EFR
(B) BRI1 and BAK1
(C) NFR1 and NFR5 (or their orthologs)
(D) TIR1 and AFB1

2007

**67.** What unique extracellular domain is present in the NFR1 and NFR5 receptors that allows binding to Nod factors?
(A) LysM (Lysin motif) domains
(B) Leucine-rich repeats (LRR)
(C) Epidermal growth factor (EGF) domains
(D) Lectin domains

2010

**68.** Following Nod factor perception, what immediate physiological event occurs in the root hair cell to trigger downstream signaling?
(A) Massive efflux of potassium leading to cell death
(B) Complete depolymerization of actin filaments
(C) Alkalinization of the vauole
(D) Rapid induction of calcium spiking in the nucleus

2019

**69.** Which channel/transporter complex located at the nuclear envelope is essential for generating the calcium spiking signature during nodulation?
(A) Cyclic nucleotide-gated channels (CNGCs)
(B) DMI1/POLLUX and CAX
(C) AHA2 plasma membrane $H^+$-ATPase
(D) NHX vacuolar exchangers

2012

**70.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The CCaMK (Calcium and Calmodulin-dependent Kinase) protein is considered the central decoder of the calcium spiking signal in the Rhizobium symbiosis pathway.
Reason (R): CCaMK directly binds to the Nod factor at the plasma membrane to sense calcium changes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.

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phyB is not effectively exposed or active in the Pr form.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**11.** What class of transcription factors do most Phytochrome Interacting Factors (PIFs) belong to?
(A) bHLH (basic helix-loop-helix) family
(B) WRKY family
(C) MYB domain proteins
(D) MADS-box family

2013

**12.** In the dark, how does the COP1-SPA1 complex primarily function in photomorphogenesis?
(A) It acts as a kinase phosphorylating phytochromes
(B) It acts as a transcriptional activator for light-induced genes
(C) It serves as an E3 ubiquitin ligase targeting photomorphogenic promoting factors for degradation
(D) It prevents the synthesis of the phytochromobilin chromophore

2023

**13.** Which key photomorphogenic transcription factor is a primary degradation target of the COP1-SPA1 complex in darkness?
(A) $PIF_1$
(B) FHY3
(C) EIN3
(D) HY5

2019

**14.** Upon light exposure, what happens to the COP1 protein to permit photomorphogenesis?
(A) It undergoes rapid proteasomal degradation
(B) It tightly binds to DNA and represses transcription
(C) It is exported from the nucleus to the cytoplasm
(D) It is phosphorylated and activated by phytochrome

2019

**15.** Consider the following statements regarding the COP1-SPA1 complex:
I. COP1 contains a RING-finger domain.
II. SPA1 acts to inhibit COP1 ligase activity in the dark.
III. The complex interacts with phyA and phyB under light conditions.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I

2023

**16.** What motif in PIF proteins mediates their physical interaction with the active Pfr form of phytochrome?
(A) bHLH domain
(B) EAR motif
(C) WD40 repeat
(D) APB (Active Phytochrome Binding) motif

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2010

**17.** Which statement accurately describes the relationship between phytochrome and COP1?
(A) Phytochrome Pfr directly inhibits COP1-SPA1 E3 ligase activity
(B) Phytochrome phosphorylates COP1 to increase its stability
(C) Phytochrome Pr transports COP1 into the nucleus
(D) Phytochrome is an obligate subunit of the COP1 complex

2002

**18.** What retrograde signaling molecule is primarily generated by chloroplasts in response to high light stress or photooxidative damage?
(A) Salicylic acid
(B) Methyl jasmonate
(C) Singlet oxygen and its derivatives
(D) Gibberellic acid

2019

**19.** In chloroplast retrograde signaling, what is the role of the GENOMES UNCOUPLED (GUN) genes?
(A) To relay signals regarding plastid functional state to the nucleus
(B) To export assembled ribosomes to the cytoplasm
(C) To transport nuclear-encoded proteins into the stroma
(D) To degrade damaged chloroplast DNA

2013

**20.** Match the GUN protein with its associated function or pathway in retrograde signaling.
List I: (Protein)
P. GUN1

(A) AP2/ERF (APETALA2/Ethylene-Responsive Factor) family
(B) bHLH family
(C) WRKY family
(D) NAC family

2013

**82.** During severe osmotic stress, what is the primary cellular function of compatible solutes like proline and βine?
(A) To act as potent enzyme inhibitors
(B) To permanently cross-link structural proteins
(C) To decrease cellular osmotic potential and maintain turgor
(D) To degrade excessive starch grains

2019

**83.** Which component of the SOS (Salt Overly Sensitive) pathway serves as a primary calcium sensor?
(A) SOS3
(B) SOS1
(C) SOS2
(D) SOS4

2007

**84.** What type of enzyme is encoded by the SOS2 gene?
(A) Plasma membrane $Na^+/H^+$ antiporter
(B) Vacuolar $H^+$-ATPase
(C) E3 ubiquitin ligase
(D) Calcineurin B-like interacting protein kinase (CIPK)

2014

**85.** How does the SOS3-SOS2 complex function to confer salt tolerance?
(A) It pumps sodium directly out of the cell via ATP hydrolysis
(B) It phosphorylates and activates SOS1, a plasma membrane $Na^+/H^+$ antiporter
(C) It seals the plasmodesmata to prevent sodium entry
(D) It degrades sodium ions into harmless molecules

2012

**86.** Which transporter is responsible for the compartmentalization of sodium ions into the vacuole to mitigate $Na^+$ toxicity in the cytosol?
(A) SOS1
(B) HKT (High-affinity $K^+$ Transporter)
(C) NHX ($Na^+/H^+$ exchanger) family proteins
(D) AHA plasma membrane proton pumps

2004

**87.** What energetic driving force enables the NHX transporters to move $Na^+$ into the vacuole against its concentration gradient?
(A) ATP hydrolysis by NHX itself
(B) The sodium gradient established by SOS1
(C) Electron transport from the mitochondria
(D) The proton gradient generated by the vacuolar $H^+$-ATPase and $H^+$-PPase

2001

**88.** What specific membrane modification anchors the SOS3 calcium sensor to the plasma membrane?
(A) N-myristoylation
(B) Palmitoylation
(C) GPI anchor attachment
(D) Phosphatidylinositol binding

2017

**89.** In addition to regulating SOS1, which vacuolar transporter is indirectly activated by the SOS2 kinase to promote cellular Na+ homeostasis?
(A) CAX1 ($Ca^{2+}/H^+$ exchanger)
(B) NHX1
(C) CHX1
(D) V-ATPase

2001

**90.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Overexpression of vacuolar NHX transporters generally enhances salt tolerance in transgenic plants.
Reason (R): It increases the efflux of toxic sodium ions from the root epidermis entirely out into the soil.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**91.** What is the structural difference between metallothioneins (MTs) and phytochelatins (PCs) regarding heavy metal detoxification?
(A) MTs are gene-encoded polypeptides; PCs are enzymatically synthesized peptides
(B) MTs are RNA molecules; PCs are proteins
(C) MTs bind exclusively to lead; PCs bind exclusively to zinc
(D) MTs are large multimeric enzymes; PCs are small lipids

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(D) A is false but R is true.

2011

**71.** What downstream transcription factor is phosphorylated and activated by CCaMK to induce nodulin gene expression?
(A) NIN (Nodule Inception)
(B) WRKY33
(C) NPR1
(D) CYCLOPS

2023

**72.** Which term describes the host-derived membrane that completely surrounds the bacteroids within the nodule cells?
(A) Symbiosome membrane (peribacteroid membrane)
(B) Tonoplast
(C) Outer mitochondrial membrane
(D) Endoplasmic reticulum membrane

2017

**73.** What is the primary function of early nodulin genes (e.g., ENOD40) during nodule organogenesis?
(A) To synthesize leghemoglobin
(B) To initiate cortical cell division and infection thread formation
(C) To provide fixed nitrogen to the plant
(D) To degrade excess bacterial cells

2017

**74.** Which specialized transcription factors regulate plant responses to abscisic acid (ABA) during drought stress?
(A) MADS-box proteins
(B) AREB/ABF (ABA-responsive element binding factors)
(C) DREB2A/2B
(D) Auxin Response Factors (ARFs)

2004

**75.** What specific cis-acting DNA element is targeted by AREB/ABF transcription factors?
(A) ABRE (ABA-responsive element)
(B) DRE/CRT (Dehydration-responsive element)
(C) W-box
(D) GCC-box

2015

**76.** In ABA-independent drought signaling, which class of transcription factors plays a primary role?
(A) AREB/ABF
(B) bZIP proteins
(C) DREB/CBF (Dehydration-responsive element binding proteins)
(D) HSFs (Heat shock factors)

2004

**77.** Which core sequence typically defines the DRE/CRT cis-element recognized by DREB proteins?
(A) ACGT
(B) TGACG
(C) CACGTG
(D) CCGAC

2001

**78.** Under non-stress conditions, how is the activity of DREB2A transcription factor primarily suppressed?
(A) Through strictly sequestering it in the mitochondria
(B) By heavy methylation of its promoter
(C) By rapid ubiquitination and proteasomal degradation mediated by DRIP1/2
(D) By irreversible binding to PIF4

2008

**79.** Which post-translational modification is required to fully activate AREB/ABF proteins in response to ABA signaling?
(A) Glycosylation by Golgi enzymes
(B) Phosphorylation by SnRK2 kinases
(C) Cleavage by caspases
(D) Farnesylation at the C-terminus

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2002

**80.** Match the drought/osmotic stress component with its function.
List I: (Component)
P. ABRE
Q. DREB2A
R. SnRK2
S. LEA proteins
List II: (Function)
1. ABA-independent transcription factor
2. Kinase responsible for phosphorylating AREBs
3. Hydrophilic proteins that protect macromolecules
4. cis-acting element in ABA-regulated promoters
(A) P-4, Q-1, R-2, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-2, Q-3, R-4, S-1

2011

**81.** Which family of transcription factors does DREB belong to?

2017

**102.** In cold acclimation, the CBF (C-repeat Binding Factor) pathway is rapidly induced. Which transcription factor directly regulates the expression of CBF genes?
(A) HsfA1
(B) DREB2A
(C) ICE1 (Inducer of CBF Expression 1)
(D) PIF3

2019

**103.** Under non-stress conditions, how is the activity of the ICE1 protein kept low to prevent untimely freezing tolerance?
(A) It undergoes rapid mRNA decay
(B) It is exported to the vacuole
(C) It remains firmly attached to the plasma membrane
(D) It is ubiquitinated by HOS1 and targeted for proteasomal degradation

2005

**104.** Which class of genes is directly upregulated by the CBF transcription factors to impart freezing tolerance?
(A) PR (Pathogenesis-Related) genes
(B) Nif (Nitrogen fixation) genes
(C) COR (Cold-Regulated) genes
(D) Nodulin genes

2017

**105.** What post-translational modification stabilizes ICE1 during cold stress, antagonizing its ubiquitination by HOS1?
(A) Phosphorylation by SnRK2
(B) SUMOylation by SIZ1
(C) Acetylation by HATs
(D) Myristoylation

2019

**106.** Which phytohormone negatively regulates the CBF-COR pathway to balance growth and cold stress response?
(A) Salicylic acid
(B) Brassinosteroid
(C) Jasmonic acid
(D) Ethylene

2015

**107.** PAMP-triggered immunity (PTI) is initiated by the recognition of Pathogen-Associated Molecular Patterns. Which molecule is a classic bacterial PAMP?
(A) Chitin
(B) Ergosterol
(C) Nod factor
(D) Flagellin (flg22)

2019

**108.** Which specific receptor-like kinase recognizes the bacterial flg22 epitope?
(A) FLS2
(B) BRI1
(C) EFR
(D) CERK1

2014

**109.** Upon binding flg22, FLS2 rapidly forms a heterodimeric complex with which essential co-receptor?
(A) TIR1
(B) NFR5
(C) GID1
(D) BAK1

NODIA

2009

**110.** Which immediate signaling event is a hallmark of the early PTI response following receptor complex formation?
(A) Massive production of gibberellins
(B) An oxidative burst (Reactive Oxygen Species production)
(C) Complete cessation of translation
(D) Depletion of cytosolic calcium

2004

**111.** Effector-triggered immunity (ETI) involves the recognition of pathogen effector proteins. Where are most plant ETI receptors localized?
(A) Extracellular matrix
(B) Plasma membrane
(C) Cytoplasm/Nucleus
(D) Vacuole

2018

**112.** The majority of disease resistance (R) proteins responsible for ETI possess which conserved structural domains?
(A) Nucleotide-binding and Leucine-rich repeat domains (NLR or NB-LRR)
(B) Transmembrane and Kinase domains
(C) LysM and Kinase domains
(D) Zinc finger and bHLH domains

2007

**113.** What is the typical physiological outcome of a robust ETI response to a biotrophic pathogen?
(A) Induction of systemic succulence
(B) Immediate abscission of the infected leaf
(C) Massive proliferation of the infected cells
(D) Rapid localized cell death known as the Hypersensitive Response (HR)

2012

**114.** According to the "Guard Hypothesis" of plant immunity, how do NLR proteins detect pathogen effectors?
(A) By directly binding to the effector protein
(B) By detecting changes in extracellular pH
(C) By sensing the modification or degradation of a host target protein manipulated by the effector
(D) By recognizing conserved PAMPs that bypassed the plasma membrane

2005

**115.** Consider the following statements regarding plant NLR proteins:
I. They can be divided into TIR-NLRs and CC-NLRs.
II. Their NB-ARC domain acts as an ATP/ADP switch regulating conformational states.
III. They exclusively recognize viral RNA genomes.
IV. Their activation leads to the establishment of systemic acquired resistance (SAR).
V. They frequently form high-molecular-weight "resistosome" complexes upon activation.
Which of the above statement(s) is/are correct?
(A) I, II, IV and V
(B) I, II and V
(C) II, III, IV and V
(D) I, II, III and IV

2010

**116.** What recently discovered mechanism describes how the CC-NLR protein ZAR1 initiates cell death upon activation?
(A) It translocates to the nucleus to bind DNA directly
(B) It acts as an RNase to cleave all host mRNA
(C) It hyperactivates the proteasome to digest the cell
(D) It forms a pentameric resistosome that acts as a calcium-permeable channel in the plasma membrane

2007

**117.** Which host enzyme is critical for amplifying the RNA interference (RNAi) signal against invading plant viruses?
(A) RNA-dependent RNA polymerase (RDR)
(B) RNA polymerase II
(C) Reverse transcriptase
(D) Poly(A) polymerase

2017

**118.** In the plant antiviral RNA silencing pathway, what is the specific role of Dicer-like (DCL) proteins?
(A) To splice viral mRNAs
(B) To methylate viral DNA
(C) To cleave double-stranded viral RNA into 21-24 nucleotide small interfering RNAs (siRNAs)
(D) To translate viral single-stranded RNA

2003

**119.** Which protein acts as the catalytic core of the RNA-induced silencing complex (RISC), slicing the target viral RNA?
(A) DCL4
(B) RDR6
(C) Argonaute (AGO)
(D) SGS3

2008

**120.** How do plant viruses primarily overcome the host's RNA silencing defense mechanism?
(A) By mutating their RNA into DNA
(B) By encoding Viral Suppressors of RNA Silencing (VSRs)
(C) By replicating exclusively within the vacuole
(D) By synthesizing thick protein capsules that DCL cannot penetrate

2024

**121.** What is the biochemical function of the well-characterized viral suppressor HC-Pro from potyviruses?
(A) It binds strongly to small double-stranded siRNAs, preventing their loading into AGO
(B) It directly degrades all AGO proteins in the cell
(C) It inhibits RNA polymerase II transcription
(D) It methylates host RDR genes

2006

**122.** Which host DCL proteins are predominantly responsible for generating the 21-nt and 22-nt siRNAs crucial for antiviral defense?
(A) DCL1 and DCL3
(B) DCL2 and DCL4
(C) DCL1 and DCL2
(D) DCL3 and DCL4

2024

**123.** Systemic RNA silencing allows plants to immunize distant tissues against a virus. How does the silencing signal travel long distances?
(A) Through the xylem
(B) Through the phloem

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(C) Via airborne volatile emissions
(D) Through continuous epidermal diffusion

2025

**124.** During plant defense signaling, the activation of MAPK cascades is universally observed. What is the sequential structure of a standard MAPK cascade?
(A) MAPKKK → MAPKK → MAPK
(B) MAPK → MAPKK → MAPKKK
(C) MAPKK → MAPKKK → MAPK
(D) MAPKKK → MAPK → MAPKK

2008

**125.** Select the correct sequential order of events in the flg22-induced PTI signaling pathway.
(A) flg22 binding → MEKK1 activation → FLS2/BAK1 dimerization → MPK3/6 activation → WRKY gene transcription
(B) flg22 binding → FLS2/BAK1 dimerization → MPK3/6 activation → MEKK1 activation → WRKY gene transcription
(C) flg22 binding → WRKY gene transcription → FLS2/BAK1 dimerization → MAPKKK activation
(D) flg22 binding → FLS2/BAK1 dimerization → MEKK1 activation → MKK4/5 activation → MPK3/6 activation → WRKY gene transcription

2005

**126.** Which two MAP kinases are most frequently identified as the terminal kinases in the primary plant defense signaling networks?
(A) MPK3 and MPK6
(B) MPK1 and MPK2
(C) MPK4 and MPK8
(D) MPK9 and MPK12

2015

**127.** How do Calcium-Dependent Protein Kinases (CDPKs) sense changes in cytosolic calcium concentrations?
(A) Via an external $Ca^{2+}$ binding subunit like calmodulin
(B) Through an intrinsic C-terminal calmodulin-like domain containing EF-hands
(C) By binding directly to the plasma membrane calcium channels
(D) Through a calcium-binding zinc finger motif

2012

**128.** What is a key regulatory difference between CDPKs and the SnRK/CBL-CIPK system in calcium signaling?
(A) CDPKs have both the kinase and the calcium sensor in a single polypeptide, unlike CIPKs
(B) CDPKs require a separate calcineurin B-like sensor, while CIPKs do not
(C) CDPKs only function in the nucleus, whereas CIPKs are strictly chloroplastic
(D) CDPKs respond to magnesium, not calcium

2009

**129.** Which critical defense response is directly mediated by the phosphorylation of Respiratory Burst Oxidase Homologs (RBOHs) by CDPKs?
(A) Synthesis of phytoalexins
(B) Callose deposition
(C) The reactive oxygen species (ROS) burst
(D) Stomatal opening

NODIA

2021

**130.** What specific class of proteins acts as the primary intracellular receptors for the phytohormone auxin?
(A) TIR1 and related Auxin Signaling F-Box (AFB) proteins
(B) Cytokinin Response 1 (CRE1)
(C) PYR/PYL/RCAR proteins
(D) GID1

2024

**131.** Within the auxin signaling pathway, what is the biochemical role of the TIR1 protein?
(A) It is a membrane-bound histidine kinase
(B) It is a transcription factor that binds auxin-response elements
(C) It is the substrate-recognizing subunit of an SCF-type E3 ubiquitin ligase
(D) It is an auxin efflux carrier

2002

**132.** In the absence of auxin, what proteins strongly repress the expression of auxin-responsive genes?
(A) Auxin Response Factors (ARFs)
(B) DELLA proteins
(C) JAZ proteins
(D) Aux/IAA proteins

2010

**133.** How do Aux/IAA proteins achieve the repression of auxin-responsive transcription?
(A) By directly binding to AuxRE DNA sequences and preventing RNA polymerase entry
(B) By forming heterodimers with activating ARFs and recruiting corepressors like TPL (TOPLESS)
(C) By actively degrading the ARF proteins
(D) By methylating the promoters of auxin-responsive genes

2003

**193.** Following the export of maltose from the chloroplast via the MEX1 transporter, which cytosolic enzyme metabolizes it for subsequent sucrose synthesis?
(A) Hexokinase
(B) A-amylase
(C) Invertase
(D) Cytocolic D-enzyme (Disproportionating enzyme 2 / DPE2)

2006

**194.** Which class of Starch Synthase (SS) is generally considered responsible for elongating the very short chains synthesized immediately after a branching event?
(A) SSII
(B) SSIII
(C) GBSS
(D) SSI

2009

**195.** Select the correct sequence describing the coordinated synthesis of an amylopectin cluster.
(A) Chain elongation by soluble SS → Chain cleavage and transfer by SBE → Trimming of disordered branches by DBE → Further elongation and crystallization
(B) Trimming of disordered branches by DBE → Chain elongation by soluble SS → Chain cleavage and transfer by SBE → Further elongation and crystallization
(C) Chain cleavage and transfer by SBE → Trimming of disordered branches by DBE → Chain elongation by soluble SS → Further elongation and crystallization
(D) Chain elongation by soluble SS → Trimming of disordered branches by DBE → Chain cleavage and transfer by SBE → Further elongation and crystallization

2007

**196.** The synthesis of transit starch occurs transiently in which specific tissue?
(A) Seed endosperm
(B) Leaf mesophyll
(C) Tuber parenchyma
(D) Root cortex

2010

**197.** Which unique metabolic pathway is essential for partitioning carbon toward starch in cereal endosperms but strictly avoided in dicot seeds?
(A) The direct import of sucrose into the amyloplast
(B) The synthesis of starch entirely in the cytosol without plastids
(C) The use of UDP-glucose by GBSS
(D) The synthesis of ADP-glucose in the cytosol followed by transport into the amyloplast via a specific adenylate translocator

2001

**198.** In response to redox changes in the stroma (linked to light reactions), how is the AGPase enzyme complex post-translationally activated?
(A) Monomerization via reduction of intermolecular disulfide bonds mediated by thioredoxin
(B) Dimerization via oxidation
(C) Phosphorylation by CDPKs
(D) Cleavage by a stromal protease

2023

**199.** What regulatory mechanism controls the transcription of the small subunit of RuBisCO (rbcS) in response to light?
(A) Mediation by light-responsive transcription factors such as HY5 binding to light-responsive elements (LREs)
(B) Direct binding of phytochrome to the rbcS promoter
(C) Light-induced opening of calcium channels exclusively
(D) Repression by the COP1 complex only in the light

2012

**200.** Which cellular condition most directly triggers the expression of the mitochondrial Alternative Oxidase (AOX) as a retrograde signaling response?
(A) Extremely high cytosolic ATP levels
(B) Saturation or restriction of the cytochrome c oxidase pathway leading to high mitochondrial ROS
(C) Plentiful supplies of nitrate in the soil
(D) Depletion of vacuolar calcium

2024

**201.** Which specific molecule acts as a rapid mobile signal transferring systemic acquired resistance (SAR) from the infected leaf to distant leaves?
(A) Azelaic acid, Pipecolic acid, and Salicylic acid derivatives
(B) Abscisic acid
(C) Cytokinins
(D) Auxins

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2019

**144.** When ABA levels rise, it binds to the PYR/PYL/RCAR receptor. What is the immediate consequence of this binding?
(A) The receptor phosphorylates SnRK2
(B) The receptor moves to the nucleus to bind ABRE elements
(C) The receptor acts as an ion channel to release calcium
(D) The receptor undergoes a conformational change that allows it to bind and inhibit PP2C phosphatases

2012

**145.** Select the correct order of events in ABA signal transduction leading to stomatal closure.
(A) ABA binding to PYR/PYL → Inhibition of PP2C → Activation of SnRK2 → Phosphorylation of SLAC1 ion channel → Efflux of anions
(B) ABA binding to PYR/PYL → Activation of PP2C → Dephosphorylation of SnRK2 → Activation of SLAC1 → Efflux of anions
(C) Activation of SnRK2 → ABA binding to PYR/PYL → Inhibition of PP2C → Phosphorylation of SLAC1 → Efflux of anions
(D) Efflux of anions → ABA binding to PYR/PYL → Inhibition of PP2C → Activation of SnRK2

2025

**146.** Which structural mechanism allows the PYR/PYL receptor to effectively capture ABA and interact with PP2C?
(A) A "gate-latch" loop closure mechanism over the hormone-binding pocket
(B) Trans-autophosphorylation of its intracellular kinase domain
(C) Disassembly of a tetrameric complex into monomers
(D) Direct cleavage by a specific protease

2019

**147.** Cytokinin signaling utilizes a pathway homologous to bacterial two-component systems. What is this pathway called?
(A) MAP kinase cascade
(B) Ubiquitination-proteasome pathway
(C) Phosphorelay cascade (Multi-step phosphorelay)
(D) Inositol triphosphate signaling

2008

**148.** The initial perception of cytokinin occurs predominantly at which cellular location?
(A) Nucleus
(B) Cytosol
(C) Endoplasmic reticulum membrane
(D) Mitochondrial inner membrane

2022

**149.** In the cytokinin signaling pathway, which family of proteins acts as the primary receptors?
(A) Ethylene Receptors (ETRs)
(B) Serine/Threonine Kinases
(C) Arabidopsis Histidine Kinases (AHKs, e.g., AHK2, AHK3, AHK4)
(D) G-protein coupled receptors (GPCRs)

2002

**150.** Following autophosphorylation of the AHK receptor upon cytokinin binding, the phosphoryl group is first transferred to an internal receiver domain and then to which mobile signaling intermediate?
(A) Arabidopsis Histidine Phosphotransfer proteins (AHPs)
(B) A-type ARR
(C) B-type ARR
(D) Calmodulin

NODIA

2003

**151.** What is the role of B-type ARRs (Arabidopsis Response Regulators) in cytokinin signaling?
(A) They are negative regulators that dampen the signal
(B) They are membrane channels that open in response to cytokinin
(C) They are transcription factors that activate cytokinin-responsive genes upon phosphorylation
(D) They act as specific proteases to degrade A-type ARRs

2025

**152.** Which class of molecules is rapidly upregulated by B-type ARRs to provide negative feedback on the cytokinin signaling pathway?
(A) C-type ARRs
(B) AHPs
(C) Cytokinin oxidases
(D) A-type ARRs

2011

**153.** Ethylene is perceived by a family of membrane-bound receptors (e.g., ETR1). In the absence of ethylene, what is the state of the receptor and its downstream signaling?
(A) The receptor is inactive; the signaling pathway is ON
(B) The receptor is active; it activates CTR1,

2005

**134.** Upon the entry of auxin into the nucleus, what molecular event directly relieves transcriptional repression?
(A) Auxin binds ARF, altering its conformation to an active state
(B) Auxin acts as "molecular glue" linking TIR1 to Aux/IAA, leading to Aux/IAA ubiquitination and degradation
(C) Auxin phosphorylates the Aux/IAA proteins, causing their export to the cytoplasm
(D) Auxin binds the promoter DNA, displacing the Aux/IAA proteins

2019

**135.** Consider the following statements regarding auxin transport and signaling:
I. PIN proteins primarily mediate polar auxin efflux.
II. AUX1/LAX proteins mediate auxin influx.
III. ARF transcription factors bind to TGTCTC auxin response elements (AuxREs).
IV. TIR1 localizes predominantly to the endoplasmic reticulum.
V. The SCF complex requires an E1 and E2 enzyme to ubiquitinate Aux/IAA.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2021

**136.** Which domain is responsible for the homo- and hetero-dimerization between ARF and Aux/IAA proteins?
(A) B3 DNA binding domain
(B) EAR motif
(C) PB1 domain (formerly domains III and IV)
(D) LRR domain

2016

**137.** Which phytohormone directly promotes the degradation of DELLA repressor proteins to stimulate plant growth?
(A) Gibberellin (GA)
(B) Auxin
(C) Cytokinin
(D) Ethylene

2025

**138.** What is the name of the soluble receptor for Gibberellin?
(A) GID1 (GIBBERELLIN INSENSITIVE DWARF 1)
(B) TIR1
(C) COI1
(D) ETR1

2006

**139.** In the GA signaling pathway, what occurs immediately after GA binds to the GID1 receptor?
(A) GID1 phosphorylates the DELLA protein
(B) GID1 translocates from the nucleus to the plasma membrane
(C) GID1 acts as a transcription factor
(D) GID1 undergoes a conformational change that allows it to interact with the DELLA protein

2018

**140.** Which F-box protein targets the GID1-GA-DELLA complex for ubiquitination and subsequent destruction by the 26S proteasome?
(A) TIR1
(B) COI1
(C) SLY1 (SLEEPY1) or SNL
(D) MAX2

2007

**141.** What is the primary molecular function of DELLA proteins when GA levels are low?
(A) They act as positive transcription factors for GA biosynthesis
(B) They repress the activity of growth-promoting transcription factors like PIFs
(C) They rapidly transport GA out of the cell
(D) They synthesize abscisic acid to maintain seed dormancy

2001

**142.** The ABA signaling network utilizes a core set of three protein classes. What are they?
(A) PYR/PYL/RCAR receptors, PP2C phosphatases, and SnRK2 kinases
(B) GID1 receptors, DELLA proteins, and SCF ligases
(C) ETR1 receptors, CTR1 kinase, and EIN2
(D) AHK kinases, AHP phosphotransfer proteins, and ARR response regulators

2001

**143.** In the absence of ABA, how is the SnRK2 kinase regulated?
(A) It is continuously targeted for degradation by the proteasome
(B) It is kept dephosphorylated and inactive by physical interaction with PP2C phosphatases
(C) It is constitutively active and phosphorylating downstream targets
(D) It is sequestered in the chloroplast stroma

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2023

**163.** How do Restorer (Rf) PPR proteins specifically suppress the CMS phenotype?
(A) By directly degrading the CMS protein via proteolysis
(B) By repairing large-scale mitochondrial genome rearrangements
(C) By synthesizing vast amounts of ATP to bypass the defect
(D) By sequence-specific binding to CMS transcripts, promoting RNA cleavage or preventing translation

2016

**164.** What structural feature defines the sequence-specific RNA binding capability of PPR proteins?
(A) Tandem arrays of 35-amino acid degenerate repeats forming a superhelical structure
(B) A zinc-finger motif
(C) A highly conserved RNA-recognition motif (RRM)
(D) A basic leucine zipper domain

2024

**165.** Which classical CMS system in maize is characterized by the T-urf13 chimeric gene and extreme susceptibility to the fungal toxin produced by Bipolaris maydis?
(A) S-type CMS
(B) Texas (T)-type CMS
(C) C-type CMS
(D) BT-type CMS

2003

**166.** Seed storage proteins are broadly classified based on their solubility properties according to the Osborne classification. Which class is soluble in aqueous alcohol?
(A) Prolamins
(B) Albumins
(C) Globulins
(D) Glutelins

2023

**167.** In cereals like maize and wheat, which class of proteins constitutes the major fraction of endosperm storage proteins?
(A) Albumins
(B) Globulins
(C) Prolamins
(D) Glutelins

2006

**168.** In legumes such as soybean and pea, which class of proteins is the predominant seed storage protein?
(A) Albumins
(B) Prolamins
(C) Glutelins
(D) Globulins

2025

**169.** What is a defining nutritional characteristic of cereal prolamins (like zein or gliadin)?
(A) They are characteristically rich in proline and glutamine but poor in lysine
(B) They are perfectly balanced in all essential amino acids
(C) They have extremely high concentrations of methionine and tryptophan
(D) They are completely insoluble in all known solvents

NODIA

2018

**170.** Which subcellular compartment is the primary site for the deposition and formation of prolamin protein bodies in cereal endosperm?
(A) The central vacuole
(B) The lumen of the rough endoplasmic reticulum (ER)
(C) The plastid stroma
(D) The apoplast

2003

**171.** Unlike prolamins, legume globulins are initially synthesized on the rough ER and then terminally deposited in which organelle?
(A) Endoplasmic reticulum
(B) Protein storage vacuoles (PSVs)
(C) Peroxisomes
(D) Mitochondria

2003

**172.** The expression of seed storage protein genes is tightly regulated at the transcriptional level. Which highly conserved cis-acting element is found in the promoters of many legume globulin genes?
(A) DRE element
(B) G-box
(C) RY repeat (CATGCATG)
(D) W-box

2024

**173.** Which seed-specific transcription factor recognizes and binds to the RY repeat to activate storage protein synthesis?
(A) bZIP transcription factors
(B) ARF transcription factors
(C) DREB proteins
(D) ABI3/B3-domain transcription factors

2014

**174.** In cereal prolamin gene promoters, the "prolamin box" (P-box) works synergistically with the GCN4-like motif (GLM) or $O_2$-box. Which transcription factor binds to the $O_2$-box in maize?
(A) VP1 (Viviparous-1)
(B) Prolamin Binding Factor (PBF)
(C) MYB domain protein
(D) Opaque-2 (a bZIP transcription factor)

2003

**175.** Select the correct sequential arrangement of events in the biosynthesis and processing of an 11S legumin-type storage protein.
(A) Cleavage of signal peptide in ER → Synthesis of preproprotein → Assembly into trimer → Transport to PSV → Cleavage into acidic/basic chains → Assembly into hexamer
(B) Synthesis of preproprotein → Cleavage of signal peptide in ER → Assembly into trimer → Transport to PSV → Cleavage into acidic/basic chains → Assembly into hexamer
(C) Synthesis of preproprotein → Transport to PSV → Cleavage of signal peptide → Cleavage into acidic/basic chains → Assembly into trimer → Assembly into hexamer
(D) Synthesis of preproprotein → Cleavage into acidic/basic chains in ER → Assembly into hexamer → Transport to PSV → Cleavage of signal peptide

2011

**176.** Which major family of transcription factors is well-documented to interact with the Prolamin box (P-box) via the PBF (Prolamin Binding Factor) protein?
(A) NAC family
(B) WRKY family
(C) MADS-box family
(D) DOF (DNA-binding with one finger) family

2025

**177.** Post-translational processing of globulins within the protein storage vacuole (PSV) involves specific cleavage. What type of enzyme catalyzes this processing?
(A) A highly specific serine protease
(B) A vacuolar processing enzyme (VPE), which is a legumain-type cysteine protease
(C) A matrix metalloproteinase
(D) An aspartic protease exclusively

2016

**178.** What structural modification occurs in the rough ER during the folding of major seed storage proteins, essential for their stability?
(A) Extensive phosphorylation
(B) Heavy methylation of arginine residues
(C) Formation of numerous intra- and inter-chain disulfide bonds
(D) Covalent attachment of phytochromobilin

2008

**179.** Starch is a semicrystalline polymer composed of two major glucan types. What are they?
(A) Amylose and amylopectin
(B) Amylose and Cellulose
(C) Amylopectin and Glycogen
(D) Maltose and Sucrose

2008

**180.** What is the structural difference between amylose and amylopectin?
(A) Amylose is exclusively β-1,4-linked; amylopectin is α-1,4-linked
(B) Amylose is essentially linear with α-1,4 linkages; amylopectin is highly branched with α-1,6 linkages
(C) Amylose is highly branched; amylopectin is strictly linear
(D) Amylose contains fructose; amylopectin contains only glucose

2013

**181.** In higher plants, what is the primary committed step in the biosynthesis of starch?
(A) The synthesis of UDP-glucose by UDP-glucose pyrophosphorylase
(B) The synthesis of glucose-6-phosphate by hexokinase
(C) The synthesis of ADP-glucose by ADP-glucose pyrophosphorylase (AGPase)
(D) The conversion of fructose-1,6-bisphosphate to fructose-6-phosphate

2008

**182.** Which compound serves as the major allosteric activator of ADP-glucose pyrophosphorylase (AGPase) in plant leaves?
(A) Inorganic phosphate (Pi)
(B) Fructose-2,6-bisphosphate
(C) AMP
(D) 3-Phosphoglycerate (3-PGA)

2008

**183.** Which compound acts as the primary allosteric inhibitor of ADP-glucose pyrophosphorylase (AGPase)?

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(A) 3-Phosphoglycerate (3-PGA)
(B) ATP
(C) Inorganic phosphate (Pi)
(D) Malate

2025

**184.** In heterotrophic tissues like the developing cereal endosperm, how does the localization of AGPase differ significantly from leaf mesophyll cells?
(A) It is entirely mitochondrial
(B) It is exclusively bound to the plasma membrane
(C) It is secreted into the apoplast
(D) A large proportion of AGPase activity is localized in the cytosol, requiring ADP-glucose transport into the amyloplast

2005

**185.** Which enzyme is uniquely responsible for the synthesis of the linear amylose chains in starch?
(A) Soluble Starch Synthase I (SSI)
(B) Granule-Bound Starch Synthase (GBSS)
(C) Starch Branching Enzyme (SBE)
(D) Isoamylase (ISA)

2014

**186.** Mutations in the gene encoding GBSS in plants like maize and rice result in what specific endosperm phenotype?
(A) Waxy phenotype (amylose-free starch)
(B) Sugary phenotype (phytoglycogen accumulation)
(C) Shrunken phenotype (drastically reduced total starch)
(D) Opaque phenotype (altered protein bodies)

2001

**187.** What is the catalytic function of Starch Branching Enzymes (SBEs)?
(A) They hydrolyze α-1,4 links to release maltose units
(B) They add ADP-glucose residues to α-1,6 branches
(C) They transform amylopectin directly into linear amylose
(D) They form α-1,6 branches via glucan chain transfer

2022

**188.** In the synthesis of highly ordered amylopectin, Debranching Enzymes (DBEs) like isoamylase play a counterintuitive but essential role. What is this role?
(A) They trim improperly placed or excessive branches to promote crystalline packing of amylopectin clusters
(B) They rapidly degrade starch during the night
(C) They generate ADP-glucose from degradation products
(D) They protect the starch granule from amylase attack

2007

**189.** A defect in the isoamylase-type debranching enzyme (e.g., in the maize sugary1 mutant) leads to the accumulation of which heavily branched, water-soluble polysaccharide instead of crystalline starch?
(A) Cellulose
(B) phytoglycogen
(C) Inulin
(D) Callose

2011

**190.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): AGPase activity in leaves is exquisitely sensitive to the ratio of 3-PGA to Pi.
Reason (R): This allosteric regulation tightly coordinates starch synthesis with the rate of photosynthetic carbon fixation and sucrose export.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**191.** During starch degradation in leaves at night, which complex primarily orchestrates the initial phosphorylation of the starch granule surface to solubilize outer chains?
(A) B-amylase (BAM)
(B) Glucan water dikinase (GWD) and Phosphoglucan water dikinase (PWD)
(C) Disproportionating enzyme (D-enzyme)
(D) Starch phosphorylase

2004

**192.** What is the major end-product of β-amylase cleavage on the linear chains of starch?
(A) Glucose
(B) Maltose
(C) Sucrose
(D) Isomaltose

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keeping the signaling pathway OFF
(C) The receptor is degraded; the signaling pathway is ON
(D) The receptor is bound to EIN2; the signaling pathway is OFF

2003

**154.** What type of enzyme is CTR1 (Constitutive Triple Response 1), a central negative regulator of the ethylene pathway?
(A) E3 ubiquitin ligase
(B) Histidine kinase
(C) Protein phosphatase 2C
(D) Serine/threonine kinase belonging to the Raf MAPKKK family

2006

**155.** Consider the following statements regarding ethylene signal transduction:
I. Ethylene binding inactivates the ETR receptors.
II. Inactivation of receptors leads to the deactivation of CTR1.
III. Deactivation of CTR1 allows the cleavage of the EIN2 C-terminal domain.
IV. The cleaved EIN2 C-terminus travels to the nucleus.
V. EIN3 is rapidly degraded in the presence of ethylene.
Which of the above statement(s) is/are correct?
(A) I, II, III and IV
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2013

**156.** In the absence of ethylene, what happens to the key transcriptional activator EIN3?
(A) It tightly binds DNA and blocks transcription
(B) It is sequestered in the vauole
(C) It is continuously targeted for proteasomal degradation by the F-box proteins $EBF_1$/EBF2
(D) It is phosphorylated and locked in an inactive state in the cytoplasm

2024

**157.** Which metal ion is an essential cofactor required for ethylene binding by the ETR1 receptor?
(A) Iron ($Fe^{2+}$)
(B) Magnesium ($Mg^{2+}$)
(C) Calcium ($Ca^{2+}$)
(D) Copper ($Cu^{+}$)

2015

**158.** Cytoplasmic Male Sterility (CMS) in plants is a maternally inherited trait. Which organelle genome is primarily responsible for specifying CMS?
(A) Mitochondrial genome
(B) Nuclear genome
(C) Chloroplast genome
(D) Amyloplast genome

2023

**159.** What molecular event is the most common cause of CMS in plant mitochondrial genomes?
(A) Extensive point mutations in highly conserved respiratory genes
(B) Complete loss of the ATP synthase complex genes
(C) Recombination events generating novel chimeric open reading frames (ORFs)
(D) Viral integration into the mitochondrial genome

2006

**160.** The toxic or disruptive proteins translated from CMS-associated chimeric ORFs typically interfere with which mitochondrial process?
(A) Inner mitochondrial membrane integrity and respiratory electron transport
(B) TCA cycle enzymes
(C) Mitochondrial ribosome assembly
(D) Import of nuclear-encoded tRNAs

2002

**161.** In agricultural hybrid seed production, CMS is utilized alongside nuclear restorer-of-fertility (Rf) genes. What is the typical function of Rf gene products?
(A) They suppress the expression or accumulation of the CMS-associated toxic mitochondrial protein
(B) They mutate the CMS mitochondrial gene back to wild-type
(C) They export the CMS protein to the vacuole
(D) They replace the mitochondria with functional paternal ones

2003

**162.** To which large protein family do the vast majority of identified restorer-of-fertility (Rf) genes belong?
(A) Kinases
(B) Chaperonins
(C) Pentatricopeptide repeat (PPR) proteins
(D) Cytochrome P450s

NODIA

2007

**202.** What is the fundamental difference in the genetic organization of nif genes between Klebsiella pneumoniae and Rhizobium species?
(A) K. pneumoniae organizes them in a clustered ~24 kb region; Rhizobium species typically have them scattered, often on large symbiotic plasmids
(B) K. pneumoniae has a single massive 20-gene operon; Rhizobium has scattered single genes
(C) K. pneumoniae nif genes lack promoters; Rhizobium nif genes have multiple promoters
(D) K. pneumoniae only uses Sigma 70; Rhizobium only uses Sigma 54

2011

**203.** During nodule development, what role do plant-derived antimicrobial peptides (NCR peptides) play in symbiosome maturation in certain legumes like Medicago?
(A) They rapidly kill and digest the bacteria to harvest nitrogen
(B) They induce terminal bacteroid differentiation, cell enlargement, and block bacterial cell division
(C) They serve as the primary carbon source for the bacteria
(D) They assemble into an oxygen-impermeable barrier

2016

**204.** Which structural domain of the NFR1/NFR5 receptors undergoes phosphorylation upon successful Nod factor binding?
(A) The extracellular LysM domain
(B) The intracellular kinase domain of NFR1
(C) The intracellular kinase domain of NFR5 (which is a pseudokinase)
(D) The transmembrane domain

2024

**205.** In heavy metal stress, the expression of genes encoding heavy metal ATPases (HMAs) is upregulated. HMA4 in Arabidopsis specifically mediates the xylem loading of which metal for root-to-shoot transport?
(A) Iron
(B) Copper
(C) Zinc and Cadmium
(D) Lead

2018

**206.** Which enzyme complex is primarily responsible for the rapid synthesis of Reactive Oxygen Species (ROS) during the PTI oxidative burst?
(A) Photosystem II
(B) Mitochondrial Complex I
(C) Plasma membrane-localized NADPH oxidases (RBOHs)
(D) Peroxisomal catalase

2015

**207.** During the induction of the CBF pathway by cold stress, what happens to membrane fluidity, and how is it perceived?
(A) Fluidity decreases (rigidification), likely perceived by mechanosensitive calcium channels
(B) Fluidity increases, perceived by lipid-binding proteins
(C) Fluidity completely halts, triggering instant cell death
(D) Fluidity relies solely on external ice crystal formation

2007

**208.** To maintain membrane fluidity during cold acclimation, what biochemical adjustment occurs in the lipid bilayer?
(A) Increased proportion of unsaturated fatty acids
(B) Increased proportion of saturated fatty acids
(C) Complete replacement of phospholipids with sterols
(D) Drastic reduction in total lipid content

2005

**209.** What common regulatory feature limits the uncontrolled hyperactivation of both MAPK and CDPK pathways during prolonged stress?
(A) Dephosphorylation by highly specific protein phosphatases (e.g., MAPK phosphatases, PP2Cs)
(B) Massive export of the kinases to the apoplast
(C) Irreversible covalent binding to the plasma membrane
(D) Complete depletion of cellular ATP

2002

**210.** Which ubiquitin ligase complex is structurally conserved between auxin signaling ($SCF^{TIR1}$), jasmonate signaling ($SCF^{COI1}$), and gibberellin signaling ($SCF^{SLY1}$)?
(A) The APC/C complex
(B) The Skp1-Cullin-F-box (SCF) complex
(C) The COP1-SPA complex
(D) The VHL complex

2007

**211.** What is the fundamental mechanism by which jasmonate (JA-Ile) activates its target genes?

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**18.** (C) Singlet oxygen and its derivatives
Chloroplasts act as environmental sensors that communicate their functional status to the nucleus through retrograde signaling. Under conditions of high light stress or photooxidative damage, the photosynthetic apparatus can produce reactive oxygen species. Singlet oxygen and its derivatives are primary signaling molecules generated in the thylakoids. These molecules trigger specific nuclear gene expression changes to help the plant adapt to stress.

**19.** (A) To relay signals regarding plastid functional state to the nucleus
The GENOMES UNCOUPLED genes play a fundamental role in coordinating the expression of nuclear genes with the functional and developmental state of the plastid. These genes encode proteins that facilitate communication from the chloroplast to the nucleus. By relaying information about plastid health and metabolic status, they ensure that the synthesis of photosynthetic components in the nucleus matches the chloroplast capacity.

**20.** (A) P-2, Q-3, R-4, S-1
Different GUN proteins perform specialized roles in retrograde signaling. GUN1 serves as a central integrator within the plastid, receiving various stress signals. GUN4 and GUN5 are involved in the tetrapyrrole biosynthesis pathway, specifically binding porphyrins and acting as subunits of magnesium chelatase. PTM is a unique plant protein located on the chloroplast envelope that functions as a membrane-bound transcription factor.

**21.** (B) Mg-protoporphyrin IX
Retrograde signaling involves several pathways, including those related to the status of chlorophyll biosynthesis. Magnesium protoporphyrin IX is a key intermediate in the tetrapyrrole pathway that has been identified as a signaling molecule. Its accumulation or flux is thought to convey information from the chloroplast to the nucleus, helping to regulate the expression of nuclear-encoded genes required for photosynthesis.

**22.** (C) Downregulation of photosynthesis-associated nuclear genes (PhANGs)
When chloroplasts experience severe photooxidative stress, they must signal the nucleus to reduce the production of photosynthetic components to prevent further damage. This retrograde communication leads to the significant downregulation of photosynthesis-associated nuclear genes. By curbing the expression of these genes, the plant limits the influx of proteins into the damaged chloroplast, facilitating recovery and prioritizing survival over active growth.

**23.** (D) PTM (PHD type transcription factor with transmembrane domain)
PTM is a unique transcription factor tethered to the chloroplast envelope by a transmembrane domain. In response to retrograde signals from the plastid interior, such as those integrated by GUN1, PTM undergoes a specific proteolytic cleavage event. This releases a C-terminal fragment into the cytosol, which then translocates into the nucleus to modulate the expression of various stress-responsive and developmental genes.

**24.** (A) ABI4
ABI4 is a versatile transcription factor originally identified in abscisic acid signaling that also plays a critical role in plastid-to-nucleus communication. It often acts downstream of multiple retrograde signaling pathways to repress the expression of photosynthesis-associated nuclear genes. By binding to specific promoter elements, ABI4 coordinates the nuclear response to the functional state of the chloroplast during development and stress.

**25.** (A) I and II
The enzyme RuBisCO is composed of subunits encoded by two different genomes within the plant cell. The large subunit is actually encoded by the chloroplast genome, while the small subunit is encoded by the nuclear genome. The nuclear-encoded small subunit is translated on cytosolic ribosomes and then imported into the chloroplast. Once inside the stroma, both subunits are assembled into the functional complex.

**26.** (B) Via an N-terminal transit peptide recognized by TOC/TIC complexes
The small subunit of RuBisCO is synthesized as a precursor in the cytosol. It contains a specific N-terminal sequence known as a transit peptide, which serves as a targeting signal. This peptide is recognized by the translocon of the outer and inner chloroplast membrane complexes, which

NODIA

(2-phosphoglycolate)
(B) Because it generates 2 molecules of ATP
(C) Because it exclusively occurs in C2 plants
(D) Because it involves only two organelles

2005

**223.** What energy penalty is associated with recovering carbon via the photorespiratory cycle?
(A) Consumption of 1 ATP and 1 NAD(P)H per oxygenation event
(B) Consumption of 2 ATP and 2 reducing equivalents (NAD(P)H or ferredoxin) per oxygenation event
(C) Total loss of all fixed carbon without energy consumption
(D) Generation of excess ATP that disrupts the chloroplast

2022

**224.** Which mitochondrial protein is essential for the reoxidation of NADH generated during glycine decarboxylation in photorespiration?
(A) Mitochondrial malate dehydrogenase (coupled with substrate shuttles)
(B) Cytochrome oxidase
(C) Alternative oxidase
(D) ATP synthase

2016

**225.** During active photorespiration, a massive flux of ammonia is released in the mitochondria. What prevents this ammonia from reaching toxic levels?
(A) Rapid export out of the leaf
(B) Efficient reassimilation by the plastidic glutamine synthetase (GS2) and Fd-GOGAT
(C) Immediate conversion into urea in the peroxisome
(D) Storage in the central vacuole

2014

**226.** What fundamental evolutionary reason explains why RuBisCO exhibits oxygenase activity alongside its vital carboxylase activity?
(A) Oxygen is required to activate the enzyme
(B) The active site cannot fully distinguish between the geometrically similar, uncharged $CO_2$ and $O_2$ molecules
(C) Oxygenation was the primary ancestral function of the enzyme
(D) Oxygenation is necessary to prevent excessive $CO_2$ accumulation

2008

**227.** In the dark, how is the activity of RuBisCO generally suppressed in many plants to prevent futile cycling?
(A) By irreversible proteolysis
(B) By complete de-carbamylation
(C) By the binding of specific tightly-bound sugar phosphate inhibitors like CA1P (2-carboxyarabinitol 1-phosphate)
(D) By targeted export from the chloroplast

2008

**228.** How does RuBisCO activase overcome the inhibition of RuBisCO by misfired or tightly bound sugar phosphates?
(A) By using ATP hydrolysis to physically alter the conformation of RuBisCO, facilitating inhibitor release
(B) By hydrolyzing the sugar phosphate
(C) By acting as a competitive inhibitor against the sugar phosphate
(D) By phosphorylating the RuBisCO active site

2006

**229.** What happens to the rate of photorespiration as leaf temperature increases significantly (e.g., above 30°C)?
(A) It decreases dramatically
(B) It remains entirely unchanged
(C) It increases relative to photosynthesis due to decreased $CO_2$ solubility and decreased specificity of RuBisCO for $CO_2$
(D) It halts completely

2013

**230.** Phytochrome signaling heavily relies on light-induced nuclear translocation. Which region of the phytochrome molecule harbors the critical Nuclear Localization Signals (NLS)?
(A) The N-terminal extension
(B) The PAS domain
(C) The C-terminal PRD (PAS-related domain) / Histidine kinase-related region
(D) The chromophore itself

2018

**231.** Under continuous far-red light (FRc), which phytochrome is the predominant active photoreceptor for the High Irradiance Response (HIR)?
(A) Phytochrome A (phyA)
(B) Phytochrome B (phyB)
(C) Phytochrome C (phyC)
(D) Phytochrome E (phyE)

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**36.** (D) It decreases its sensitivity to malate inhibition
Phosphorylation of phosphoenolpyruvate carboxylase is a critical regulatory mechanism in C4 photosynthesis. This modification occurs on a specific serine residue near the N-terminus of the protein. The primary effect of this phosphorylation is to alter the enzyme's sensitivity to feedback inhibition. Specifically, the phosphorylated form of the enzyme is significantly less sensitive to inhibition by malate, allowing continued carbon fixation.

**37.** (B) Its activity is primarily controlled by strictly de novo protein synthesis and degradation
The kinase responsible for phosphorylating phosphoenolpyruvate carboxylase is regulated in a unique manner compared to many other signaling kinases. Its activity is primarily controlled by the amount of protein present in the cell. The enzyme undergoes rapid de novo synthesis in response to light signals and is quickly degraded in the dark, ensuring that the target enzyme is only activated during the day.

**38.** (C) Mesophyll cytosol
C4 plants utilize a specialized leaf anatomy and enzymatic organization to minimize photorespiration. The enzyme phosphoenolpyruvate carboxylase performs the initial step of capturing inorganic carbon. This enzyme is specifically localized within the cytosol of the mesophyll cells. By capturing bicarbonate in the cytosol, it facilitates the formation of four-carbon acids that are later moved to the bundle sheath chloroplasts.

**39.** (D) It ensures PEPC remains highly active despite rising cytosolic malate levels
During the daytime, C4 plants actively fix carbon dioxide, leading to the accumulation of malate in the mesophyll cells. Malate normally acts as a potent feedback inhibitor of phosphoenolpyruvate carboxylase. To maintain high rates of photosynthesis, the plant phosphorylates the enzyme. This modification ensures that the carboxylase remains highly active and continues to fix carbon, even as cytosolic malate levels rise.

**40.** (B) P-2, Q-3, R-1, S-1
The photorespiratory pathway is a complex metabolic process that spans multiple organelles. Glycolate oxidase is a key enzyme localized in the peroxisome. Serine hydroxymethyltransferase is found within the mitochondria, where it participates in the conversion of glycine. Conversely, both phosphoglycolate phosphatase and glycerate kinase are situated in the chloroplast, where they handle the initial and final steps of the recycling pathway.

**41.** (B) 2-Phosphoglycolate
RuBisCO is a bifunctional enzyme that can react with either carbon dioxide or oxygen. When it uses oxygen as a substrate, it performs an oxygenase reaction instead of carboxylation. This reaction results in the production of one molecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate. The latter is a toxic compound that must be recycled through the energy-intensive photorespiratory pathway.

**42.** (B) Mitochondrion
Photorespiration involves the movement of metabolites through several cellular compartments. After being processed in the chloroplast and peroxisome, two molecules of glycine are transported into the mitochondrion. Within the mitochondrial matrix, these glycine molecules are converted into one molecule of serine. This reaction is a central part of the pathway and results in the release of carbon dioxide and ammonia.

NODIA

**43.** (A) M-protein (magnesium binding)
The glycine decarboxylase complex is a massive multienzyme system located in the mitochondrial matrix of plant cells. It is composed of four distinct proteins known as the P, H, T, and L proteins. Each protein has a specific catalytic role, such as binding pyridoxal phosphate or utilizing lipoamide. Unlike these components, an M-protein is not a recognized subunit of this particular enzyme complex.

**44.** (C) Ammonia
During the photorespiratory cycle, the glycine decarboxylase complex catalyzes a critical reaction in the mitochondria. This process involves the conversion of two glycine molecules into one serine molecule. A significant consequence of this reaction is the release of several byproducts into the mitochondrial matrix, most notably carbon dioxide and a molecule of ammonia, which must be subsequently reassimilated by the plant.

(C) Rapid export of siRNAs into the soil
(D) Integration of siRNAs into the host genome as permanent genes

2014

**242.** In systemic acquired resistance (SAR), which phytohormone accumulates massively in systemic uninfected tissues to prime defenses?
(A) Auxin
(B) Gibberellin
(C) Salicylic acid (SA)
(D) Cytokinin

2008

**243.** The master regulator of SAR signaling is NPR1. How does SA influence NPR1 activity?
(A) SA alters cellular redox state and binds to NPR receptors, causing NPR1 oligomers to dissociate into active monomers that enter the nucleus
(B) SA triggers the degradation of NPR1
(C) SA explicitly exports NPR1 to the apoplast
(D) SA blocks NPR1 from interacting with transcription factors

2014

**244.** Once in the nucleus, which family of transcription factors does NPR1 interact with to induce PR (Pathogenesis-Related) gene expression?
(A) MYB
(B) WRKY and TGA (bZIP) transcription factors
(C) DREB
(D) ARF

2017

**245.** In the context of seed storage proteins, what characterizes the mature structure of a typical 11S globulin (e.g., glycinin)?
(A) A single highly disordered polypeptide
(B) A hexameric complex composed of six acidic-basic heterodimers held by disulfide bonds
(C) A long, unbranched α-helix
(D) A cyclic peptide

2011

**246.** What post-transcriptional mechanism generates extensive polymorphism in cereal prolamin gene families, complicating their study?
(A) Massive RNA splicing variations from a single gene
(B) They are encoded by large multi-gene families that arose via extensive tandem duplication and mutation
(C) RNA editing by PPR proteins
(D) Viral reverse transcription

2015

**247.** To prevent early germination, abscisic acid (ABA) strongly induces the expression of which seed-specific transcription factor to maintain dormancy?
(A) ABI3
(B) $PIF_1$
(C) LEC1
(D) FUS3

2002

**248.** During starch biosynthesis, the enzyme GBSS requires processivity. Which molecule acts as an essential cofactor for GBSS activity within the starch granule?
(A) Malto-oligosaccharides
(B) Malate
(C) Proteolipid / Protein Targeting to Starch (PTST)
(D) Flavin adenine dinucleotide (FAD)

2017

**249.** Which of the following best explains why amylose synthesis lags behind amylopectin synthesis during cereal endosperm development?
(A) GBSS transcription only initiates at seed maturity
(B) Amylose is synthesized inside the growing amylopectin matrix; therefore, a substantial amylopectin matrix must form first before GBSS can act optimally
(C) ADP-glucose levels are too low early in development
(D) Isoamylase degrades early amylose

2008

**250.** How does the plant selectively degrade starch during the night while avoiding spontaneous degradation during the day?
(A) Through strict transcriptional regulation (synthesis of degradative enzymes only at night)
(B) Through redox-regulation and the physical separation of degradative enzymes alongside allosteric controls
(C) By pumping all degradative enzymes into the vacuole during the day
(D) Starch granules are physically coated with impenetrable lipids during the day

**********

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(A) By directly phosphorylating transcription factors
(B) By promoting the $SCF^{COI1}$-mediated degradation of JAZ repressor proteins
(C) By inhibiting the proteasome
(D) By activating a receptor histidine kinase

2007

**212.** In the context of seed storage proteins, what is a "protein body"?
(A) A complex of ribosomes actively translating storage proteins
(B) An insoluble aggregate of storage proteins sequestered in specialized organelles (ER derived or PSV)
(C) A specific chaperone that folds globulins
(D) A viral particle accumulated in seeds

2018

**213.** The opaque-2 mutation in maize significantly improves the nutritional quality of the grain. What is the primary biochemical consequence of this mutation?
(A) Increased total starch content
(B) Decreased synthesis of α-zeins, leading to a compensatory increase in lysine-rich proteins
(C) Massive overproduction of β-carotene
(D) Complete loss of amylopectin

2012

**214.** Which form of nitrogen is primarily transported out of the nitrogen-fixing bacteroid into the host plant cytosol?
(A) Nitrate ($NO_3^-$)
(B) Nitrite ($NO_2$-)
(C) Ammonia ($NH_3$ / $NH_4^+$)
(D) Glutamine

2022

**215.** Legumes of tropical origin (e.g., soybean) export assimilated nitrogen from the nodule primarily in which form?
(A) Ureides (Allantoin and Allantoic acid)
(B) Amides (Asparagine and Glutamine)
(C) Unmodified Ammonia
(D) Free amino acids like Leucine

2004

**216.** Temperate legumes (e.g., pea, clover) typically export fixed nitrogen from nodules as:
(A) Ureides
(B) Amides (mainly Asparagine and Glutamine)
(C) Uric acid
(D) Nitric oxide

2018

**217.** In the Rhizobium-legume symbiosis, what carbon compound is the primary energetic fuel supplied by the plant to the bacteroids?
(A) Dicarboxylic acids (e.g., malate, succinate)
(B) Sucrose
(C) Glucose
(D) Starch

2004

**218.** The MoFe-protein of nitrogenase contains a complex metal cluster required for electron transfer to the FeMoco. What is this cluster named?
(A) The Rieske center
(B) The P-cluster ([8Fe-7S])
(C) The Q-cycle intermediate
(D) Plastoquinone

2004

**219.** During the operation of the C4 photosynthetic pathway in NADP-ME type plants (e.g., maize), which 4-carbon acid is transported from mesophyll to bundle sheath cells?
(A) Malate
(B) Aspartate
(C) Oxaloacetate
(D) Pyruvate

NODIA

2023

**220.** After decarboxylation in the bundle sheath of NADP-ME C4 plants, which 3-carbon compound returns to the mesophyll cells to regenerate PEP?
(A) Pyruvate
(B) Alanine
(C) 3-Phosphoglycerate
(D) Glyceraldehyde 3-phosphate

2012

**221.** In C4 photosynthesis, how does the anatomical separation of enzymes (Kranz anatomy) suppress photorespiration?
(A) By concentrating $CO_2$ at the site of RuBisCO in the bundle sheath, strongly favoring carboxylase over oxygenase activity
(B) By removing oxygen entirely from the plant leaf
(C) By directly inhibiting the oxygenase activity of RuBisCO via an allosteric effector
(D) By lacking the RuBisCO enzyme entirely

2012

**222.** The photorespiratory pathway is often termed the C2 cycle. Why?
(A) Because the first unique product of the oxygenase reaction is a 2-carbon compound

**9.** (B) Interacting with and regulating transcriptional regulators
Phytochromes do not generally bind directly to DNA sequences or act as histones. Instead, they function by entering the nucleus and interacting with a variety of transcriptional regulators, such as Phytochrome Interacting Factors and various E3 ubiquitin ligases. Through these protein-protein interactions, phytochromes modulate the stability and activity of transcription factors, thereby controlling the expression of numerous light-responsive genes.

**10.** (A) Both A and R are true and R is the correct explanation of A.
Phytochrome B remains predominantly in the cytoplasm of dark-grown seedlings because its inactive Pr form does not facilitate nuclear entry. The nuclear localization signal within the protein is effectively masked or inactive until red light absorption occurs. This light-induced conformational change exposes the signal, allowing the active Pfr form to be recognized by importin proteins and transported into the nucleus.

**11.** (A) bHLH (basic helix-loop-helix) family
Phytochrome Interacting Factors constitute a specific group of proteins that play a major role in light-mediated development. Most of these factors belong to the basic helix-loop-helix family of transcription factors. They typically act as repressors of photomorphogenesis in the dark by binding to specific DNA motifs, such as G-boxes, in the promoters of genes involved in plant growth.

**12.** (C) It serves as an E3 ubiquitin ligase targeting photomorphogenic promoting factors for degradation
The COP1-SPA complex is a central repressor of photomorphogenesis that functions primarily in the dark. It operates as an E3 ubiquitin ligase, identifying and tagging positive regulators of light signaling, such as the transcription factor HY5, with ubiquitin molecules. This process ensures these promoting factors are degraded by the 26S proteasome, preventing the initiation of light-induced development in darkness.

**13.** (D) HY5
HY5 is a basic leucine zipper transcription factor that promotes photomorphogenesis by activating light-inducible genes. In the absence of light, its accumulation is prevented by the COP1-SPA complex. This E3 ubiquitin ligase complex binds to HY5 in the nucleus and targets it for proteasomal degradation. Consequently, HY5 levels remain low in the dark, and light-responsive developmental programs are suppressed.

**14.** (C) It is exported from the nucleus to the cytoplasm
To permit the initiation of photomorphogenesis upon light exposure, the repressive activity of COP1 must be neutralized. This is achieved through the light-induced nuclear export of the COP1 protein. Once translocated to the cytoplasm, COP1 can no longer access its nuclear targets, such as HY5. This reduction in nuclear COP1 concentration allows positive regulators to accumulate and activate light-responsive genes.

**15.** (B) I and III
The COP1-SPA complex is a multi-subunit E3 ubiquitin ligase. COP1 contains a RING-finger domain essential for its catalytic activity in transferring ubiquitin. While SPA1 is often associated with enhancing COP1 activity in the dark, the entire complex physically interacts with active phytochromes like phyA and phyB under light conditions. This interaction is crucial for the light-mediated inhibition of COP1.

NODIA

**16.** (D) APB (Active Phytochrome Binding) motif
Phytochrome Interacting Factors utilize specific conserved motifs to recognize and bind to the active forms of phytochromes. While many PIFs contain a Phytochrome B-binding motif, the Active Phytochrome Binding motif is specifically responsible for mediating the physical interaction with the Pfr form. This binding is a prerequisite for the subsequent phosphorylation and degradation of the transcription factors in the nucleus.

**17.** (A) Phytochrome Pfr directly inhibits COP1-SPA1 E3 ligase activity
Phytochromes and the COP1-SPA complex have an antagonistic relationship in light signaling. When plants are exposed to light, the active Pfr form of phytochrome enters the nucleus and directly interacts with the COP1-SPA complex. This interaction leads to the dissociation or inhibition of the E3 ligase activity, preventing the degradation of photomorphogenic promoters and allowing the plant to develop normally.

# ANSWERS

**1.** (A) GAF domain
Phytochromes are complex photoreceptors consisting of a protein component and a light-absorbing pigment molecule. The N-terminal region of the apophytochrome contains a GAF domain, which is essential for holoprotein formation. This specific domain functions as a lyase, facilitating the covalent attachment of the phytochromobilin chromophore to a conserved cysteine residue, thereby enabling light sensing and signal transduction.

**2.** (C) Cysteine
The formation of functional phytochrome requires the covalent attachment of a linear tetrapyrrole chromophore, known as phytochromobilin, to the apoprotein. This linkage occurs specifically through a thioether bond formed with a conserved cysteine residue located within the GAF domain. This residue provides the sulfur atom necessary for the covalent binding that allows the molecule to perceive light signals.

**3.** (A) Cis to trans isomerization at the C15 double bond
Phytochromes exist in two interconvertible forms, Pr and Pfr, which respond to different light wavelengths. Upon the absorption of red light by the Pr form, the phytochromobilin chromophore undergoes a fundamental structural change. This process involves a Z-to-E or cis-to-trans isomerization of the double bond at the C15 position between the C and D pyrrole rings, initiating signal transduction.

**4.** (B) 660 nm
The red-light-absorbing form of phytochrome, known as Pr, is biologically inactive and predominantly found in dark-grown plants. Exposure to red light, specifically around the 660 nm wavelength, triggers a conformational change that converts Pr into the active Pfr form. This active state is responsible for initiating various photomorphogenic responses, such as seed germination, leaf expansion, and inhibiting hypocotyl elongation.

**5.** (B) Cell nucleus
Phytochrome A is a light-sensitive protein that plays a vital role in plant development. In its inactive form, it remains in the cytoplasm. However, upon perception of light and conversion to the active Pfr state, the protein undergoes rapid translocation into the cell nucleus. Once inside the nucleus, it interacts with various transcription factors to regulate the expression of light-responsive genes.

**6.** (C) FHY1 and FHL
The nuclear import of the active Pfr form of phytochrome A is a critical step in light signaling. Unlike phytochrome B, which contains an intrinsic nuclear localization signal, phytochrome A requires specialized transporter proteins. FHY1 and its homolog FHL specifically bind to the Pfr form of phytochrome A, facilitating its movement from the cytoplasm through the nuclear pore complex.

**7.** (D) Rapid phosphorylation and subsequent degradation of PIFs
Phytochrome Interacting Factors are negative regulators of photomorphogenesis that accumulate in the dark. When active phytochromes enter the nucleus, they physically interact with these factors. This interaction leads to the rapid phosphorylation of the transcription factors, marking them for ubiquitination and subsequent degradation by the 26S proteasome. This removal allows the plant to initiate light-induced growth and developmental programs.

**8.** (A) It promotes hypocotyl elongation in dark-grown seedlings
The active Pfr form of phytochrome is essential for mediating de-etiolation responses when seedlings are exposed to light. Its physiological roles include the initiation of seed germination, the stimulation of leaf expansion, and the regulation of flowering time. Contrary to dark-grown behavior, Pfr actually inhibits hypocotyl elongation, ensuring that the plant allocates resources toward leaf development and photosynthetic machinery.

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facilitate the transport of the protein into the stroma where the signal peptide is removed.

**27.** (A) Eight large and eight small subunits (L8S8)
RuBisCO is a massive multimeric enzyme complex found in the stroma of higher plant chloroplasts. Its functional quaternary structure consists of sixteen subunits in total. Specifically, it is organized as a hexadecamer featuring eight large subunits and eight small subunits, commonly denoted as the L8S8 form. This complex arrangement is essential for its catalytic activity in fixing atmospheric carbon dioxide.

**28.** (C) GroEL/GroES-like chaperonin complex (Cpn60/Cpn10)
The large subunit of RuBisCO is synthesized within the chloroplast and requires specialized assistance to achieve its functional three-dimensional shape. This folding process is facilitated by a chaperonin complex in the stroma, which is homologous to the bacterial GroEL/GroES system. Known as Cpn60 and Cpn10, these proteins provide a protected environment that allows the large subunit to fold correctly before assembly.

**29.** (D) $RAF_1$ (RuBisCO Accumulation Factor 1)
The assembly of the RuBisCO holoenzyme from its individual large and small subunits is a complex, multi-step process. In addition to general chaperonins, specific ancillary proteins called assembly factors are required. RuBisCO Accumulation Factor 1 is one such essential protein that interacts with the large subunits to facilitate their proper oligomerization into the final L8S8 structure within the chloroplast stroma.

**30.** (C) A is true but R is false.
RuBisCO activase is an essential enzyme that maintains RuBisCO in a catalytically active state by removing inhibitory sugar phosphates from its active site. However, the reasoning provided is incorrect. RuBisCO activase does not provide carbon dioxide to the enzyme. Instead, it uses energy from ATP hydrolysis to induce conformational changes in RuBisCO, ensuring the enzyme remains ready for carbon fixation.

**31.** (B) Carbamylation
Before RuBisCO can catalyze the fixation of carbon dioxide, it must undergo a specific activation process. This involves the covalent attachment of a non-substrate carbon dioxide molecule to the epsilon-amino group of a conserved lysine residue. This post-translational modification, known as carbamylation, is essential for creating the metal-binding site required for the enzyme's catalytic function in the Calvin cycle.

**32.** (D) Magnesium ($Mg^{2+}$)
The activation of the RuBisCO enzyme is a two-step process occurring in the chloroplast stroma. Following the carbamylation of a specific lysine residue, a divalent metal ion must bind to the site to complete the active center. Magnesium is the essential ion that coordinates with the carbamate group, stabilizing the complex and facilitating the subsequent binding and catalysis of the substrate.

NODIA

**33.** (A) Phosphoenolpyruvate carboxylase (PEPC)
In C4 plants, carbon fixation is divided between two different cell types to increase efficiency. The initial step occurs in the cytosol of mesophyll cells, where atmospheric carbon is first captured. This reaction is catalyzed by the enzyme phosphoenolpyruvate carboxylase. It converts phosphoenolpyruvate and bicarbonate into oxaloacetate, a four-carbon acid, which is then transported to the bundle sheath cells.

**34.** (C) $HCO_3^-$ and Phosphoenolpyruvate
Phosphoenolpyruvate carboxylase is the primary enzyme responsible for the initial fixation of inorganic carbon in C4 and CAM plants. Unlike RuBisCO, which uses gaseous carbon dioxide, this enzyme specifically utilizes the bicarbonate ion as its carbon source. It catalyzes the carboxylation of phosphoenolpyruvate to produce oxaloacetate, playing a vital role in the carbon-concentrating mechanism that reduces photorespiration.

**35.** (C) II and III
The activity of phosphoenolpyruvate carboxylase in C4 plants is tightly regulated to coordinate with the light reactions of photosynthesis. The enzyme is allosterically activated by glucose-6-phosphate, which signals an abundance of triose phosphates. Additionally, its activity is modulated by phosphorylation. In the light, the activity of a specific kinase increases, leading to the phosphorylation and subsequent activation of the enzyme.

2003

**232.** Why is phytochrome A uniquely suited to mediate the far-red High Irradiance Response (FR-HIR)?
(A) Its Pfr form is highly stable in the light
(B) It is rapidly degraded in the Pfr form, causing a continuous flux of newly synthesized, hypersensitive Pr molecules acting dynamically under FR
(C) It only absorbs blue light
(D) It cannot undergo photoreversibility

2014

**233.** The shade avoidance response (e.g., enhanced stem elongation in crowded canopies) is primarily triggered by which environmental cue perceived by phytochromes?
(A) A low Red to Far-Red (R:FR) ratio
(B) A high Red to Far-Red (R:FR) ratio
(C) Total darkness
(D) High blue light intensity

2023

**234.** During shade avoidance, a drop in active Pfr levels leads to the stabilization of which transcription factors to promote elongation growth?
(A) HY5
(B) PIF4 and PIF5
(C) HSFs
(D) DREBs

2022

**235.** The interaction of active phytochrome (Pfr) with PIFs promotes PIF degradation. What enzyme specifically phosphorylates PIF prior to its ubiquitination in some cases?
(A) Phototropin kinase
(B) SnRK2
(C) Casein Kinase 2 (CK2) or directly by Phytochrome itself functioning as a weak kinase
(D) MAPKKK

2022

**236.** In the COP1-SPA E3 ligase complex, what is the specific role of the SPA (Suppressor of phya-105) proteins?
(A) They act as the catalytic RING-finger
(B) They bind to DNA
(C) They are essential co-factors that enhance the substrate-targeting and ubiquitination activity of COP1
(D) They synthesize the ubiuitin molecules

2005

**237.** Which statement represents a core principle of retrograde signaling from plastids to the nucleus?
(A) Retrograde signals coordinate nuclear gene expression with plastid developmental status and environmental stresses
(B) The nucleus ignores the physiological state of plastids entirely
(C) Plastids actively secrete transcription factors into the environment
(D) The signal strictly involves the physical transport of entire ribosomes

2019

**238.** In identifying the pathways of retrograde signaling, experiments using the inhibitor norflurazon are common. What does norflurazon specifically inhibit to generate a retrograde signal?
(A) RuBisCO
(B) Phytoene desaturase in the carotenoid biosynthesis pathway, leading to photo-oxidative bleaching
(C) ATP synthase
(D) Starch synthase

2012

**239.** The chloroplast-localized GUN1 protein is considered a central integrator of multiple retrograde signals. While its exact molecular function is complex, what family of proteins does it belong to?
(A) Pentatricopeptide repeat (PPR) proteins with a Small MutS-Related (SMR) domain
(B) Kinases
(C) E3 ubiquitin ligases
(D) Cytochrome P450s

2024

**240.** During severe heat stress, denatured proteins can aggregate. Which chaperone family specifically uses ATP to untangle these rigid aggregates (e.g., functioning as a disaggregase)?
(A) sHSP
(B) HSP70
(C) HSP100 (e.g., ClpB)
(D) Calnexin

2023

**241.** What is the fundamental mechanism of gene silencing by small interfering RNAs (siRNAs) in plant viral defense?
(A) Sequence-specific base pairing of the siRNA to the viral mRNA, guiding AGO to cleave the target
(B) Direct binding of siRNAs to viral capsids

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**45.** (B) II
The photorespiratory cycle is an essential metabolic pathway that helps plants manage the products of the RuBisCO oxygenase reaction. It involves complex interactions between the chloroplast, peroxisome, and mitochondrion and consumes both ATP and reducing equivalents. Importantly, the pathway is not perfectly efficient; it only recovers about seventy-five percent of the carbon, meaning some carbon is lost as carbon dioxide.

**46.** (C) Through the GS/GOGAT cycle in the chloroplast
In the course of photorespiration, a large amount of ammonia is generated within the mitochondria. Because ammonia is toxic to the cell, it must be rapidly reassimilated. This is primarily achieved through the action of the glutamine synthetase and glutamate synthase cycle, which is localized in the chloroplast. This pathway efficiently incorporates the nitrogen back into amino acids, preserving cellular resources.

**47.** (B) Hydroxypyruvate reductase
The peroxisome is a critical site for several reactions within the photorespiratory pathway. After serine is converted into hydroxypyruvate through an aminotransferase reaction, the next step involves a reduction process. The enzyme hydroxypyruvate reductase uses reducing equivalents, typically in the form of NADH, to convert hydroxypyruvate into glycerate. The resulting glycerate is then transported back to the chloroplast to rejoin the Calvin cycle.

**48.** (D) Fe-protein and MoFe-protein
Biological nitrogen fixation is catalyzed by the nitrogenase enzyme complex found in certain microorganisms. This complex consists of two highly conserved metalloproteins that work together. The first component is the dinitrogenase reductase, also known as the iron protein. The second component is the dinitrogenase, commonly referred to as the molybdenum-iron protein, which contains the active site for the reduction of dinitrogen.

**49.** (A) It acts as an ATP-dependent reductase, transferring electrons to the MoFe-protein
Within the nitrogenase complex, the iron protein serves as a specialized electron donor for the molybdenum-iron protein. It functions as an ATP-dependent reductase. During the catalytic cycle, the iron protein binds ATP and undergoes a conformational change that lowers its reduction potential. This allows it to transfer high-energy electrons to the molybdenum-iron protein, which then uses them to reduce nitrogen.

**50.** (C) A is true but R is false.
The nitrogenase enzyme is extremely sensitive to the presence of molecular oxygen, which causes rapid and irreversible inactivation of the complex. This sensitivity presents a major challenge for nitrogen-fixing organisms. However, oxygen does not act as a competitive inhibitor at the active site. Instead, it chemically reacts with and destroys the metal clusters within the enzyme, rendering it permanently non-functional.

**51.** (A) In the FeMoco (Iron-Molybdenum cofactor) of the MoFe-protein
The reduction of dinitrogen into ammonia is a chemically demanding process that occurs within the larger component of the nitrogenase complex. Specifically, the catalytic active site is located buried within the molybdenum-iron protein. This site consists of a unique and complex metal cluster known as the iron-molybdenum cofactor. It is here that nitrogen molecules bind and receive electrons to be reduced.

NODIA

**52.** (C) 16
Nitrogen fixation is an energetically expensive biological process due to the extreme stability of the triple bond in dinitrogen. For every molecule of nitrogen reduced to two molecules of ammonia, a significant amount of energy is required. The nitrogenase enzyme complex minimally hydrolyzes sixteen molecules of ATP to drive the transfer of the eight electrons necessary for the full reduction reaction.

**53.** (B) nifB, nifE, nifN, and nifV
The assembly of the complex iron-molybdenum cofactor within the nitrogenase enzyme requires a specialized set of biosynthetic genes. While some genes encode the structural components of the enzyme, others are dedicated to cofactor synthesis. Genes such as nifB, nifE, nifN, and nifV are primarily responsible for the complex biochemical steps involved in building and inserting the FeMoco into the MoFe-protein.

**54.** (C) A carbon atom
Recent high-resolution structural studies have revealed a surprising feature of the iron-

membrane acts as a critical interface for the exchange of nutrients and signals during nitrogen fixation.

**73.** (B) To initiate cortical cell division and infection thread formation
Early nodulin genes are among the first host genes to be expressed during the symbiotic interaction with Rhizobium. These genes play fundamental roles in the morphological changes required for nodule formation. Their primary functions include the initiation of cell divisions in the root cortex to form the nodule primordium and the coordination of the growth of infection threads through root hairs.

**74.** (B) AREB/ABF (ABA-responsive element binding factors)
When plants experience drought stress, they accumulate the hormone abscisic acid to coordinate defensive responses. The transcriptional response to this hormone is primarily mediated by a specific group of basic leucine zipper proteins. Known as AREB or ABF transcription factors, these proteins are activated by phosphorylation and bind to specific elements in the promoters of genes that help the plant tolerate dehydration.

**75.** (A) ABRE (ABA-responsive element)
The regulation of gene expression by abscisic acid during water deficit depends on the presence of specific DNA sequences in the promoters of target genes. The most important of these cis-acting elements is the ABA-responsive element, commonly referred to as the ABRE. This sequence is the direct binding site for AREB/ABF transcription factors, allowing for the precise activation of the stress-protective pathway.

**76.** (C) DREB/CBF (Dehydration-responsive element binding proteins)
Plants have evolved multiple signaling pathways to respond to drought and dehydration, some of which do not require the hormone abscisic acid. In these ABA-independent pathways, the DREB or CBF class of transcription factors plays a primary role. These proteins belong to the AP2/ERF family and are responsible for activating a wide array of stress-responsive genes that provide protection against osmotic stress.

**77.** (D) CCGAC
ABA-independent stress signaling often involves the recognition of a specific cis-acting DNA element known as the Dehydration-Responsive Element or C-repeat. This element is found in the promoters of many genes that are induced by drought, high salinity, and cold. The core sequence that defines this element and is recognized by the DREB class of transcription factors is typically the pentanucleotide CCGAC.

**78.** (C) By rapid ubiquitination and proteasomal degradation mediated by DRIP1/2
To prevent the activation of stress responses under favorable environmental conditions, the levels of key transcription factors like DREB2A are strictly regulated. This is achieved through a process of continuous protein degradation. In the absence of stress, E3 ubiquitin ligases known as DRIP1 and DRIP2 identify DREB2A and target it for destruction by the proteasome, ensuring the protein does not accumulate.

**79.** (B) Phosphorylation by SnRK2 kinases
The activation of AREB/ABF transcription factors is a critical step in the abscisic acid signaling pathway. For these proteins to effectively bind to their target DNA elements and initiate transcription, they must undergo a specific post-translational modification. This process involves phosphorylation by SnRK2-type protein kinases, which are themselves activated when ABA levels rise in response to environmental stress such as drought.

**80.** (A) P-4, Q-1, R-2, S-3
Several components work together to coordinate the plant response to osmotic stress. ABRE is the DNA element that mediates ABA-dependent responses, while DREB2A is a major transcription factor in the ABA-independent pathway. The SnRK2 kinases serve as essential signaling links by phosphorylating target proteins. Finally, LEA proteins represent a large group of hydrophilic molecules that physically protect other proteins during dehydration.

**81.** (A) AP2/ERF (APETALA2/Ethylene-Responsive Factor) family
DREB transcription factors are central players in the response to environmental stresses such as cold and drought. These proteins are characterized by the presence of a conserved DNA-binding domain that defines their family.

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roots secrete specific secondary metabolites that serve as signaling molecules. These compounds, primarily flavonoids and isoflavonoids, act as chemoattractants for the bacteria. They also trigger the expression of bacterial nodulation genes, which is the first step in forming a nodule.

**64.** (D) NodD
In Rhizobium bacteria, the perception of plant-derived signals is mediated by the NodD protein. NodD is a constitutively expressed transcription factor that belongs to the LysR family. When it binds to specific flavonoids released by the legume root, it undergoes a conformational change. This allows it to bind to conserved DNA sequences, called nod boxes, activating the transcription of other nodulation genes.

**65.** (A) I, II and IV
Nod factors are essential signaling molecules produced by bacteria to initiate nodulation. Chemically, they are lipo-chitooligosaccharides consisting of a backbone of several N-acetyl-D-glucosamine residues linked by β-1,4 bonds. They feature a specific fatty acyl chain attached to the non-reducing end of the sugar chain. These structural features are critical for their recognition by the specific receptors on host root hairs.

**66.** (C) NFR1 and NFR5 (or their orthologs)
For a legume to respond to the presence of symbiotic bacteria, it must perceive the secreted Nod factors. This recognition is performed by a set of specialized receptor-like kinases located at the plasma membrane of root hair cells. Proteins such as NFR1 and NFR5, or their orthologs in various species, bind directly to the Nod factors to initiate the signaling cascade.

**67.** (A) LysM (Lysin motif) domains
The ability of plant receptors to specifically bind to the chitin-like backbone of Nod factors depends on their extracellular structure. The NFR1 and NFR5 receptors contain multiple LysM domains in their extracellular portion. These domains are well-known for their capacity to interact with carbohydrate molecules containing N-acetylglucosamine, providing the specificity required for the initial molecular recognition of the bacterial signal.

NODIA

**68.** (D) Rapid induction of calcium spiking in the nucleus
Shortly after the perception of Nod factors by receptors on the root hair surface, a sophisticated intracellular signaling process begins. One of the earliest and most distinctive physiological markers of this pathway is the induction of periodic oscillations in calcium concentration. This phenomenon, known as calcium spiking, occurs primarily within and around the nucleus and is essential for activating downstream genes.

**69.** (B) DMI1/POLLUX and CAX
The generation of the calcium spiking signal in the nucleus during nodulation requires the coordinated action of several proteins. Specialized ion channels and transporters located on the nuclear envelope are responsible for the flux of calcium ions. Components such as DMI1 and POLLUX are essential for this process, as they help regulate the electrical potential and ion movement needed to produce spikes.

**70.** (C) A is true but R is false.
The CCaMK protein is a central regulator in the symbiotic signaling pathway, acting as a decoder that translates nuclear calcium oscillations into gene expression changes. While it is essential for the process, it is not a primary receptor for Nod factors. Instead, CCaMK is localized to the nucleus, where it senses and responds to the calcium signal generated by other components.

**71.** (D) CYCLOPS
In the symbiotic signaling pathway, the calcium and calmodulin-dependent kinase CCaMK must activate downstream targets to promote nodule formation. One of its primary substrates is a protein known as CYCLOPS. Upon being phosphorylated by CCaMK, CYCLOPS undergoes a conformational change that allows it to function as a transcription factor, directly inducing the expression of early nodulation genes in the nucleus.

**72.** (A) Symbiosome membrane (peribacteroid membrane)
Inside the cells of a mature root nodule, the nitrogen-fixing bacteria are not free in the cytoplasm. Instead, they are housed within specialized compartments. Each bacterium, or group of bacteria, is completely enclosed by a host-derived membrane known as the symbiosome membrane or peribacteroid membrane. This

contains an extracellular leucine-rich repeat domain that binds directly to the flg22 molecule. This binding event triggers the activation of various intracellular signaling pathways.

**109.** (D) BAK1
Upon binding its ligand flg22, the FLS2 receptor must interact with a co-receptor to initiate signaling. This essential partner is another receptor-like kinase called BAK1. The formation of the FLS2-BAK1 heterodimer allows the intracellular kinase domains of both proteins to phosphorylate each other. This activation of the receptor complex is a fundamental requirement for the subsequent induction of the immune responses.

**110.** (B) An oxidative burst (Reactive Oxygen Species production)
The activation of the FLS2-BAK1 receptor complex by bacterial flagellin leads to a series of rapid physiological changes. One of the most immediate and characteristic hallmarks of this immune response is the production of reactive oxygen species. This oxidative burst occurs within minutes of perception and serves both as a direct antimicrobial defense and as a signaling mechanism to activate further defensive genes.

**111.** (C) Cytoplasm/Nucleus
Plants have evolved a second layer of immunity to detect pathogen effectors that have been delivered into the cell. The receptors responsible for this detection, known as Resistance proteins, are primarily localized within the intracellular space. They can be found in the cytoplasm or the nucleus, where they monitor the state of host proteins or bind directly to the invading fungal or bacterial effectors.

**112.** (A) Nucleotide-binding and Leucine-rich repeat domains (NLR or NB-LRR)
The majority of plant disease resistance proteins that recognize pathogen effectors belong to a large and conserved family. These proteins are characterized by two primary structural domains. The first is a central nucleotide-binding domain that acts as a molecular switch. The second is a C-terminal leucine-rich repeat domain, which is typically involved in the specific recognition of the pathogen-derived molecules or their effects.

**113.** (D) Rapid localized cell death known as the Hypersensitive Response (HR)
When an intracellular resistance protein successfully detects a pathogen effector, it triggers a robust immune response. For biotrophic pathogens, which require living host tissue, the most common outcome is the hypersensitive response. This involves the rapid and programmed death of the infected cells and their immediate neighbors. By sacrificing these cells, the plant effectively creates a localized barrier that prevents the pathogen spreading.

**114.** (C) By sensing the modification or degradation of a host target protein manipulated by the effector
The guard hypothesis describes a common mechanism by which plant resistance proteins detect pathogen activity. Instead of binding directly to the effector molecule, the resistance protein acts as a guard that monitors the integrity of a specific host target protein. When a pathogen effector modifies or degrades this target to facilitate infection, the guard protein detects the change and triggers the immune response.

**115.** (A) I, II, IV and V
Plant NLR proteins are diverse receptors that manage intracellular immunity. They are categorized based on their N-terminal domains into TIR-NLRs or CC-NLRs and use a nucleotide-binding domain as a switch. While they primarily detect protein effectors rather than viral RNA, their activation leads to the establishment of systemic immunity. Many of these proteins assemble into large complexes called resistosomes to initiate signaling.

**116.** (D) It forms a pentameric resistosome that acts as a calcium-permeable channel in the plasma membrane
The CC-NLR protein ZAR1 provides a landmark model for understanding how resistance proteins function. Upon activation, ZAR1 molecules assemble into a pentameric complex known as a resistosome. This high-molecular-weight structure translocates to the plasma membrane, where it inserts to form a pore. This pore acts as a calcium-permeable channel, causing a massive influx of calcium into the cell, which ultimately triggers the hypersensitive response.

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Specifically, DREBs belong to the AP2/ERF family of transcription factors, which is unique to plants and involved in a wide variety of developmental and stress-related processes.

**82.** (C) To decrease cellular osmotic potential and maintain turgor
Under severe osmotic stress, plants accumulate high concentrations of small, organic molecules known as compatible solutes. Common examples include the amino acid proline and the quaternary ammonium compound glycine βine. These molecules do not interfere with cellular metabolism but serve to significantly decrease the cellular osmotic potential. This allows the plant to absorb more water and maintain its turgor pressure.

**83.** (A) SOS3
The salt overly sensitive pathway is the primary mechanism for maintaining ionic balance during salinity stress. This pathway is initiated by the perception of an increase in cytosolic calcium levels. SOS3 is the specialized calcium-sensing protein that detects this signal. It contains EF-hand motifs that bind calcium, causing a conformational change that allows it to interact with and activate downstream signaling components.

**84.** (D) Calcineurin B-like interacting protein kinase (CIPK)
The SOS2 gene encodes an essential protein kinase that functions within the salt tolerance pathway. It is a member of the calcineurin B-like interacting protein kinase family. SOS2 is specifically activated by its interaction with the calcium sensor SOS3. Once active, this kinase plays a vital role in regulating the activity of ion transporters that remove excess sodium from the plant cell.

**85.** (B) It phosphorylates and activates SOS1, a plasma membrane $Na^+/H^+$ antiporter
The SOS3-SOS2 complex is a critical regulatory unit that manages the cellular response to high salt concentrations. Upon activation by calcium, the complex targets the SOS1 protein, which is a sodium/proton antiporter located on the plasma membrane. The SOS2 kinase phosphorylates SOS1, significantly increasing its activity. This leads to the efficient export of toxic sodium ions from the cytosol into the apoplast.

**86.** (C) NHX ($Na^+/H^+$ exchanger) family proteins
To protect the cytoplasm from the damaging effects of high sodium levels, plants can sequester these ions into the large central vacuole. This process of compartmentalization is mediated by a family of transporters known as NHX proteins. These sodium/proton exchangers use the energy of a proton gradient to move sodium into the vacuole, thereby lowering its concentration in the metabolic compartments.

**87.** (D) The proton gradient generated by the vacuolar $H^+$-ATPase and $H^+$-PPase
The transport of sodium ions into the vacuole by NHX exchangers is an active process that requires an energetic driving force. This force is provided by a proton gradient established across the tonoplast. Specialized enzymes, namely the vacuolar $H^+$-ATPase and the $H^+$-pyrophosphatase, use chemical energy to pump protons into the vacuole. This creates the electrochemical potential that NHX transporters utilize.

**88.** (A) N-myristoylation
For the SOS3 calcium sensor to effectively relay signals within the salt tolerance pathway, it must be properly localized. This protein is anchored to the plasma membrane through a specific lipid modification. A process known as N-myristoylation occurs at the N-terminus of the SOS3 protein, attaching a fatty acid chain that embeds into the lipid bilayer. This localization is essential for its function.

**89.** (B) NHX1
While the primary target of the SOS2 kinase is the plasma membrane sodium exporter SOS1, it also influences other cellular transporters to ensure overall sodium balance. Evidence suggests that SOS2 can indirectly or directly modulate the activity of vacuolar transporters, such as NHX1. By promoting the activity of these exchangers, the SOS signaling pathway enhances the sequestration of sodium into the vacuole.

**90.** (C) A is true but R is false.
Increasing the expression of vacuolar NHX transporters is a well-established strategy for enhancing salt tolerance in crops. These proteins facilitate the removal of sodium from the cytosol by pumping it into the vacuole. However, the reasoning provided is incorrect. The function of these transporters is to sequester sodium within

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molybdenum cofactor, which is the active site of nitrogenase. At the center of this large metal-sulfur cluster, there is a single, centrally coordinated atom. This atom has been identified as a carbon atom. Its presence is essential for the structural integrity and the unique catalytic properties of the enzyme complex.

**55.** (A) I and II
Nitrogen-fixing organisms have evolved various strategies to protect the oxygen-sensitive nitrogenase enzyme. Legumes utilize leghemoglobin to buffer oxygen levels within the nodules. Some free-living bacteria, like Azotobacter, maintain low internal oxygen through exceptionally high rates of respiration. In contrast, while cyanobacteria use heterocysts, these specialized cells actually lack Photosystem II to avoid oxygen production, rather than lacking Photosystem I.

**56.** (A) NifA
In the bacterium Klebsiella pneumoniae, the expression of genes involved in nitrogen fixation is tightly controlled at the transcriptional level. NifA serves as the master regulatory protein in this system. It acts as a transcriptional activator that specifically binds to the promoters of various nif operons. By interacting with the RNA polymerase complex, NifA initiates the synthesis of the nitrogenase enzyme components.

**57.** (B) High oxygen and high combined nitrogen
The activity of the transcriptional activator NifA is modulated by a sensor protein called NifL. This regulation ensures that nitrogen fixation only occurs when environmental conditions are favorable. NifL directly inhibits NifA when it detects high levels of molecular oxygen or high concentrations of combined nitrogen in the environment. This mechanism prevents the cell from wasting energy on nitrogen fixation.

**58.** (B) It activates the transcription of the nifLA operon under nitrogen-limiting conditions
Nitrogen regulation in many bacteria involves a sophisticated signaling hierarchy. When combined nitrogen levels are low, the NtrC protein becomes phosphorylated and active. Once activated, NtrC functions as a transcriptional activator that specifically targets the promoter of the nifLA operon. By promoting the expression of this operon, NtrC indirectly triggers the entire nitrogen fixation pathway through the production of NifA.

**59.** (A) A heme-based histidine kinase that acts as an oxygen sensor
In the symbiotic relationship between Rhizobium and legumes, the regulation of nitrogen fixation genes is governed by an oxygen-sensing system. FixL is a critical component of this two-component regulatory pathway. It is a membrane-bound, heme-containing histidine kinase that directly monitors oxygen concentrations. Under low-oxygen conditions typical of the nodule interior, FixL remains active and initiates a signaling cascade.

**60.** (B) P-2, Q-4, R-1, S-3
Symbiotic nitrogen fixation requires a coordinated regulatory network. FixL acts as the primary sensor kinase that detects low oxygen levels. It subsequently phosphorylates FixJ, a response regulator that activates the expression of further regulatory genes. NifA then serves as the direct transcriptional activator for structural nif genes. This entire process requires a specialized alternative sigma factor, RpoN, for successful transcription.

NODIA

**61.** (B) -12 and -24 sequences
The transcription of genes involved in nitrogen fixation requires a specialized form of the RNA polymerase holoenzyme. This enzyme utilizes an alternative sigma factor known as Sigma 54 or RpoN. Unlike the standard house-keeping sigma factor that recognizes -10 and -35 promoter elements, the RpoN-containing polymerase specifically identifies and binds to conserved sequences located at the -12 and -24 positions.

**62.** (C) An enhancer-binding AAA+ ATPase domain
NifA belongs to a class of transcriptional activators known as enhancer-binding proteins. These proteins typically bind to DNA sequences far upstream of the transcription start site. To initiate the transition of the RNA polymerase-Sigma 54 complex from a closed to an open state, NifA utilizes a specialized AAA+ ATPase domain. This domain provides the energy needed to remodel the complex.

**63.** (B) Flavonoids and isoflavonoids
The establishment of symbiosis between legumes and Rhizobium bacteria begins with a molecular dialogue in the rhizosphere. The host plant

trimerization is essential for the proteins to acquire high-affinity DNA-binding capability, allowing them to recognize and bind to the heat shock elements present in the promoters of target genes.

**100.** (A) P-4, Q-2, R-3, S-1
Different families of heat shock proteins perform specialized functions in protein maintenance. Small HSPs bind denatured proteins in an ATP-independent manner to prevent aggregation. HSP70 proteins facilitate refolding in conjunction with co-chaperones. HSP90 is involved in the final maturation of signaling proteins. Finally, the HSP100 family uses ATP to untangle and disaggregate proteins that have already formed clumps during severe heat stress.

**101.** (A) HSE (Heat shock element)
The transcriptional activation of genes in response to heat stress is mediated by the binding of Heat Shock Factors to specific DNA sequences. These sequences are known as Heat Shock Elements and are typically found in multiple copies within the promoters of responsive genes. The assembly of trimeric factors on these elements is the key step in triggering the production of protective chaperones.

**102.** (C) ICE1 (Inducer of CBF Expression 1)
Cold acclimation is a process by which plants increase their freezing tolerance through the activation of the CBF signaling pathway. The expression of the CBF genes is itself under tight control. A MYC-type transcription factor called ICE1 is a master regulator of this process. ICE1 binds directly to the promoters of CBF genes, inducing their expression and thereby initiating the cold-responsive pathway.

**103.** (D) It is ubiquitinated by HOS1 and targeted for proteasomal degradation
The activity of the ICE1 protein must be carefully regulated to prevent the inappropriate activation of cold-responsive genes. Under non-stress conditions, ICE1 is targeted for degradation to keep its levels low. An E3 ubiquitin ligase called HOS1 performs this role by identifying ICE1 and tagging it for destruction by the proteasome. This ensures that the freezing tolerance pathway is only activated when necessary.

**104.** (C) COR (Cold-Regulated) genes
The CBF transcription factors are central components of the plant response to low temperatures. Once synthesized, these proteins bind to the promoters of a specialized group of target genes known as Cold-Regulated genes. The products of these genes, which include various protective proteins and enzymes for metabolite synthesis, work together to stabilize cellular structures and membranes, thereby conferring increased freezing tolerance.

**105.** (B) SUMOylation by SIZ1
When plants are exposed to cold stress, the ICE1 protein must be stabilized to activate the downstream signaling pathway. This is achieved through a specific post-translational modification called SUMOylation. The E3 SUMO ligase SIZ1 attaches a SUMO molecule to ICE1, which prevents its ubiquitination by HOS1. This stabilization allows ICE1 to accumulate in the nucleus and promote the expression of the CBF transcription factors.

**106.** (D) Ethylene
Plants must balance their limited resources between growth and stress responses. In the context of cold stress, several hormones influence the CBF-mediated pathway. Ethylene has been identified as a negative regulator of this system. By suppressing the expression of the CBF genes and their downstream targets, ethylene helps to moderate the freezing tolerance response, ensuring that the plant does not overinvest in defense.

**107.** (D) Flagellin (flg22)
Plants possess an innate immune system that detects the presence of potential pathogens through the recognition of conserved microbial molecules. These are known as Pathogen-Associated Molecular Patterns. A classic example is flagellin, the primary protein component of bacterial flagella. Specifically, a highly conserved 22-amino acid epitope from flagellin, called flg22, is recognized by plant surface receptors to initiate immunity.

**108.** (A) FLS2
The recognition of the bacterial flagellin epitope flg22 is a critical step in initiating plant defense. This molecular perception is carried out by a specific receptor-like kinase located on the plasma membrane. Known as FLS2, this receptor

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WRKY transcription factors, inducing defense genes.

**126.** (A) MPK3 and MPK6
In the complex network of plant immune signaling, two specific mitogen-activated protein kinases frequently serve as the central convergence points. MPK3 and MPK6 are the most prominent terminal kinases in several defense-related cascades. Upon activation by various upstream signals, they phosphorylate numerous substrates, including transcription factors and enzymes involved in the synthesis of defensive metabolites and reactive oxygen species.

**127.** (B) Through an intrinsic C-terminal calmodulin-like domain containing EF-hands
Calcium-dependent protein kinases are unique signaling proteins that combine a catalytic kinase domain and a calcium sensor in a single polypeptide. They possess an intrinsic C-terminal calmodulin-like domain containing several EF-hand motifs. When cytosolic calcium concentrations rise during stress, these motifs bind calcium ions, inducing a conformational change that removes an auto-inhibitory segment from the active site, thereby activating the enzyme.

**128.** (A) CDPKs have both the kinase and the calcium sensor in a single polypeptide, unlike CIPKs
A fundamental distinction exists between different calcium-sensing kinase families in plants. Calcium-dependent protein kinases are monomeric proteins where both the regulatory calcium-binding domain and the catalytic kinase domain reside within the same polypeptide chain. In contrast, the SnRK/CBL-CIPK system requires two separate proteins: a calcineurin B-like sensor that must physically interact with and activate a separate kinase to relay the calcium signal.

**129.** (C) The reactive oxygen species (ROS) burst
The rapid production of reactive oxygen species during plant defense is a tightly regulated process. The enzymes responsible for this oxidative burst, known as Respiratory Burst Oxidase Homologs, are located at the plasma membrane. These enzymes are directly activated by calcium-dependent protein kinases. Upon sensing a calcium signal, the kinases phosphorylate the N-terminal region of the RBOH proteins, triggering the immediate release of superoxide.

**130.** (A) TIR1 and related Auxin Signaling F-Box (AFB) proteins
The perception of the phytohormone auxin occurs within the cell through a specialized group of proteins. TIR1 and its related auxin signaling F-box proteins serve as the primary intracellular receptors. These proteins are components of an E3 ubiquitin ligase complex. When auxin is present, it binds directly to the TIR1/AFB protein, enabling the subsequent steps of the hormone signaling pathway that lead to gene expression.

**131.** (C) It is the substrate-recognizing subunit of an SCF-type E3 ubiquitin ligase
Within the auxin signaling network, the TIR1 protein performs a critical biochemical role as a hormone receptor. It functions as the substrate-recognizing subunit of an SCF-type E3 ubiquitin ligase complex. The binding of auxin to TIR1 increases its affinity for specific repressor proteins. This allows the complex to tag these repressors for degradation, which is the key mechanism for activating auxin-responsive genes.

**132.** (D) Aux/IAA proteins
In the absence of auxin, the plant prevents the expression of growth-related genes using a family of short-lived repressor proteins. Known as Aux/IAA proteins, these molecules remain in the nucleus and physically interact with transcription factors. By binding to these factors, the Aux/IAA proteins block their ability to activate gene expression, ensuring that the auxin response is kept in an inactive state.

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**133.** (B) By forming heterodimers with activating ARFs and recruiting corepressors like TPL (TOPLESS)
Aux/IAA proteins achieve transcriptional repression through several mechanisms. They contain a conserved domain that allows them to form heterodimers with Auxin Response Factor transcription factors. Once bound to an ARF, the Aux/IAA protein recruits a corepressor called TOPLESS. This complex modification of the chromatin or direct interference with the transcriptional machinery effectively silences the target genes until auxin levels rise and trigger repression relief.

**117.** (A) RNA-dependent RNA polymerase (RDR)
Plants use RNA interference as a primary defense against viral infections. For this mechanism to be effective, the initial silencing signal must be amplified to overcome the rapid replication of the virus. This amplification is performed by a host enzyme called RNA-dependent RNA polymerase. It uses single-stranded viral RNA templates to synthesize double-stranded RNA, providing more substrate for the production of silencing molecules.

**118.** (C) To cleave double-stranded viral RNA into 21-24 nucleotide small interfering RNAs (siRNAs)
In the plant antiviral silencing pathway, the detection of double-stranded RNA is a key step. Specialized enzymes known as Dicer-like proteins are responsible for processing this RNA. They act as molecular scissors, cleaving the long double-stranded viral RNA into small fragments called small interfering RNAs. These small RNAs then serve as the guide molecules that allow the host to target and destroy viral genetic material.

**119.** (C) Argonaute (AGO)
The final and most critical stage of the RNA silencing pathway involves the destruction of viral RNA. This process is carried out by a large multi-protein complex called the RNA-induced silencing complex. The catalytic core of this complex is an Argonaute protein. Using a small interfering RNA as a guide, the Argonaute protein identifies the complementary viral mRNA and uses its endonuclease activity to slice it.

**120.** (B) By encoding Viral Suppressors of RNA Silencing (VSRs)
To successfully infect their hosts, many plant viruses have evolved strategies to counteract the RNA silencing defense mechanism. They produce specialized proteins known as viral suppressors of RNA silencing. These proteins can interfere with the host's defense at various stages, such as by binding to small RNAs to prevent their use or by directly inhibiting the activity of Dicer-like or Argonaute proteins.

**121.** (A) It binds strongly to small double-stranded siRNAs, preventing their loading into AGO
The HC-Pro protein produced by potyviruses is a well-studied example of a viral suppressor of RNA silencing. Its primary mechanism of action involves the sequestration of small interfering RNAs. By binding tightly to these double-stranded molecules, HC-Pro prevents them from being loaded into the Argonaute proteins. This effectively blocks the formation of the silencing complexes and allows the virus to replicate and spread.

**122.** (B) DCL2 and DCL4
Different Dicer-like proteins in plants are specialized for producing small RNAs of various sizes. In the context of antiviral defense, DCL4 and DCL2 are the most important members of this family. DCL4 is typically the primary enzyme responsible for generating 21-nucleotide siRNAs, while DCL2 acts redundantly to produce 22-nucleotide siRNAs. Together, they ensure the production of the silencing signals needed to target viral genomes.

**123.** (B) Through the phloem
RNA silencing is not restricted to the initially infected cells; it can spread throughout the entire plant to provide systemic immunity. This long-distance movement of silencing signals is achieved through the plant's vascular system. Small RNAs and other signaling intermediates are transported through the phloem, following the source-to-sink flow of photoassimilates. This allows distant tissues to prepare their defenses before the virus arrives.

**124.** (A) MAPKKK → MAPKK → MAPK
Mitogen-activated protein kinase cascades are universal signaling modules that relay information from receptors to the nucleus. These cascades are organized in a standard three-tier hierarchy of protein kinases. A MAP kinase kinase kinase first activates a MAP kinase kinase by phosphorylation. The activated second kinase then phosphorylates the final MAP kinase, which typically translocates into the nucleus to modify transcriptional activity.

**125.** (D) flg22 binding → FLS2/BAK1 dimerization → MEKK1 activation → MKK4/5 activation → MPK3/6 activation → WRKY gene transcription
The signaling pathway initiated by the perception of bacterial flagellin is highly organized. It begins with the binding of flg22 to the FLS2-BAK1 receptor complex. This leads to the activation of a MAP kinase cascade, where MEKK1 triggers MKK4 or MKK5. These, in turn, activate the terminal kinases MPK3 and MPK6. Finally, these kinases enter the nucleus to phosphorylate

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the cell, not to export it into the soil from the roots.

**91.** (A) MTs are gene-encoded polypeptides; PCs are enzymatically synthesized peptides
Plants use two distinct classes of small, sulfur-rich molecules to detoxify heavy metals. Metallothioneins are traditional proteins that are directly encoded by genes and synthesized by ribosomes. In contrast, phytochelatins are short peptides that are not produced through translation. Instead, they are synthesized enzymatically from glutathione by a specific enzyme in response to the presence of metal ions in the cell.

**92.** (C) Cysteine
Metallothioneins are specialized proteins involved in the homeostasis and detoxification of various metal ions. Their remarkable ability to bind metals like copper and zinc is due to their unique amino acid composition. These proteins are exceptionally rich in cysteine residues. The sulfhydryl groups provided by these cysteines are essential for forming stable coordination complexes with the metal ions, preventing their toxicity.

**93.** (D) Glutathione (GSH)
Phytochelatins are critical peptides that bind and sequester heavy metals in plant cells. The synthesis of these molecules is carried out by the enzyme phytochelatin synthase. This enzyme uses glutathione as its direct substrate. By linking multiple glutathione molecules together and removing the terminal glycine, the enzyme produces the characteristic repeating structure of phytochelatins, which are used to chelate metals.

**94.** (C) Through direct post-translational activation by heavy metal ions binding to the enzyme
The regulation of phytochelatin synthesis must be rapid to protect the cell from sudden metal toxicity. This is achieved through the direct control of the enzyme phytochelatin synthase. Rather than relying on slow transcriptional changes, the enzyme is constitutively present but inactive. It becomes post-translationally activated only when heavy metal ions bind directly to the enzyme, triggering the immediate production of phytochelatins.

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**95.** (B) Central vacuole
After heavy metal ions are chelated by phytochelatins in the cytoplasm, the resulting complexes must be safely stored to prevent damage to cellular processes. The primary destination for these complexes is the large central vacuole. Transporting these metal-peptide units into the vacuole effectively isolates the toxic metals from sensitive metabolic machinery, representing a final and crucial step in the detoxification pathway.

**96.** (D) ABCC-type ABC transporters
The movement of metal-phytochelatin complexes into the vacuole is an active transport process that requires specific membrane proteins. These proteins are members of the ATP-binding cassette family of transporters, specifically the ABCC subclass. These transporters utilize the energy from ATP hydrolysis to move the bulky complexes across the tonoplast, ensuring that the toxic metals are successfully compartmentalized away from the cytosol.

**97.** (A) Zinc
Transition metals like zinc are essential micronutrients that must be carefully managed to avoid deficiency or toxicity. The MTP1 transporter is a member of the cation diffusion facilitator family and is localized to the vacuolar membrane. Its primary function is to pump excess zinc into the vacuole. This activity is vital for maintaining zinc homeostasis and for protecting the plant under conditions of high zinc availability.

**98.** (C) They are released from repressing complexes containing HSP70/HSP90
The heat shock response is initiated by the activation of Heat Shock Factors that are already present in the cell. Under normal temperatures, these factors are kept in an inactive, monomeric state through their association with chaperone proteins like HSP70 and HSP90. When temperatures rise, these chaperones are recruited to bind to other unfolding proteins, releasing the factors to initiate transcription.

**99.** (A) Trimers
Upon their release from inhibitory chaperone complexes during heat stress, Heat Shock Factors must undergo a structural transformation to become active. They assemble into a specific oligomeric form known as a trimer. This

**134.** (B) Auxin acts as "molecular glue" linking TIR1 to Aux/IAA, leading to Aux/IAA ubiquitination and degradation
The entry of auxin into the nucleus triggers a rapid molecular event that relieves the repression of target genes. Auxin acts as a molecular glue by binding into a pocket at the interface between the TIR1 receptor and the Aux/IAA repressor protein. This stabilizes their interaction, allowing the SCF complex to ubiquitinate the Aux/IAA protein, marking it for rapid degradation by the proteasome.

**135.** (A) I, II, III and V
Auxin signaling and transport are highly coordinated processes. PIN proteins and AUX1/LAX carriers manage the directional flow of the hormone between cells. Inside the cell, ARF transcription factors bind to specific DNA motifs to regulate gene expression. The degradation of repressor proteins involves an SCF ubiquitin ligase complex, which requires E1 and E2 enzymes to function. Importantly, the TIR1 receptor is nuclear.

**136.** (C) PB1 domain (formerly domains III and IV)
The interaction between Auxin Response Factors and Aux/IAA repressor proteins is fundamental to the control of auxin signaling. This protein-protein interaction is mediated by a shared structural motif. Formerly known as domains three and four, this region is now called the PB1 domain. It allows for the formation of various homo- and heterodimers, which determine the transcriptional state of auxin-responsive genes depending on hormone levels.

**137.** (A) Gibberellin (GA)
Gibberellins are vital plant hormones that promote stem elongation and other growth-related processes. Their primary mode of action is to relieve growth repression. In the absence of the hormone, DELLA proteins act as powerful suppressors of growth. When gibberellin levels increase, the hormone triggers a signaling cascade that leads to the rapid ubiquitination and proteasomal degradation of these DELLA repressors, thereby promoting growth.

**138.** (A) GID1 (GIBBERELLIN INSENSITIVE DWARF 1)
The perception of gibberellins in plant cells is mediated by a specific soluble receptor. Known as GID1, this protein is primarily located in the nucleus. GID1 contains a hormone-binding pocket that allows it to sense and bind to biologically active gibberellins. This binding event is the first step in the signaling pathway that eventually leads to the removal of growth-inhibiting proteins from the nucleus.

**139.** (D) GID1 undergoes a conformational change that allows it to interact with the DELLA protein
When gibberellin binds to the GID1 receptor, it triggers a significant structural change in the protein. A flexible lid or gate loop closes over the hormone-binding pocket. This conformational change creates a new surface on the GID1 protein that has a high affinity for DELLA repressor proteins. The resulting formation of a GA-GID1-DELLA complex is essential for the subsequent degradation of the repressor.

**140.** (C) SLY1 (SLEEPY1) or SNL
The degradation of DELLA proteins is the central regulatory event in gibberellin signaling. Once the DELLA protein is recruited into a complex with the GA-bound GID1 receptor, it is recognized by a specific E3 ubiquitin ligase. The F-box protein responsible for this recognition is called SLY1 in Arabidopsis or SNL in rice. This interaction leads to the ubiquitination and destruction of the DELLA protein.

**141.** (B) They repress the activity of growth-promoting transcription factors like PIFs
Under conditions where gibberellin levels are low, the DELLA proteins accumulate and exert a powerful inhibitory effect on plant development. Their primary molecular function is to act as repressors by physically interacting with and sequestering various growth-promoting transcription factors, such as Phytochrome Interacting Factors. By preventing these factors from binding to their target DNA sequences, DELLA proteins effectively halt stem elongation.

**142.** (A) PYR/PYL/RCAR receptors, PP2C phosphatases, and SnRK2 kinases
The core signaling pathway for abscisic acid is composed of three essential types of proteins. The process begins with the perception of the hormone by a family of receptors called PYR, PYL, or RCAR. These receptors interact with type 2C protein phosphatases, which are negative regulators. The final components are SnRK2-type protein kinases, which activate

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the downstream stress-responsive transcription factors and ion channels.

**143.** (B) It is kept dephosphorylated and inactive by physical interaction with PP2C phosphatases
In the absence of abscisic acid, the plant must keep the stress response pathway inactive. This is achieved through the continuous inhibition of SnRK2 kinases. Specifically, PP2C phosphatases bind directly to these kinases and keep them in a dephosphorylated and inactive state. As long as the phosphatases are active, they prevent the kinases from phosphorylating their downstream targets, such as transcription factors or ion channels.

**144.** (D) The receptor undergoes a conformational change that allows it to bind and inhibit PP2C phosphatases
When the levels of abscisic acid increase in response to stress, the hormone binds to its intracellular receptors. This binding event triggers a conformational change in the receptor that allows it to physically interact with and inhibit the PP2C phosphatases. By locking the phosphatases in an inactive state, the receptor effectively removes the inhibition on the SnRK2 kinases, allowing the signaling pathway to proceed.

**145.** (A) ABA binding to PYR/PYL → Inhibition of PP2C → Activation of SnRK2 → Phosphorylation of SLAC1 ion channel → Efflux of anions
The signaling cascade leading to stomatal closure begins with ABA binding to the PYR/PYL receptors. This inhibits PP2C phosphatases, which otherwise keep the SnRK2 kinases inactive. Once released from inhibition, SnRK2 kinases become active and phosphorylate downstream targets, such as the SLAC1 anion channel. This phosphorylation triggers a massive efflux of anions, leading to the loss of guard cell turgor and closure.

**146.** (A) A "gate-latch" loop closure mechanism over the hormone-binding pocket
The PYR/PYL family of abscisic acid receptors utilizes a sophisticated structural mechanism for hormone perception. These proteins feature a central binding pocket covered by a flexible loop structure. When ABA enters the pocket, the gate and latch loops close over the hormone, stabilizing it. This closure creates a surface that is specifically recognized by the PP2C phosphatases, allowing the receptor-hormone complex to inhibit them.

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**147.** (C) Phosphorelay cascade (Multi-step phosphorelay)
Cytokinin signaling in plants is remarkably similar to the two-component systems found in bacteria but has evolved into a more complex form. It is characterized as a multi-step phosphorelay cascade. This process involves the transfer of a phosphoryl group from a histidine kinase receptor through several intermediate proteins, eventually reaching response regulators in the nucleus that control the expression of cytokinin-responsive genes.

**148.** (C) Endoplasmic reticulum membrane
While many hormone receptors are located on the plasma membrane, the primary perception of cytokinin occurs at a different cellular location. Studies have shown that the cytokinin receptors are predominantly localized within the membrane of the endoplasmic reticulum. This localization suggests that the hormone must enter the cell to be perceived, highlighting the importance of specialized transporters in coordinating the overall cytokinin response.

**149.** (C) Arabidopsis Histidine Kinases (AHKs, e.g., AHK2, AHK3, AHK4)
The initial sensing of cytokinin molecules is performed by a specific family of proteins. These are known as Arabidopsis Histidine Kinases, such as AHK2, AHK3, and AHK4. These membrane-bound receptors contain an extracellular domain that binds the hormone. Upon perception, the receptor undergoes autophosphorylation on a conserved histidine residue, initiating the multi-step phosphorelay that transmits the signal into the cell nucleus.

**150.** (A) Arabidopsis Histidine Phosphotransfer proteins (AHPs)
Following the autophosphorylation of a cytokinin receptor, the signal must be transmitted from the endoplasmic reticulum to the nucleus. This is achieved by mobile signaling intermediates called Arabidopsis Histidine Phosphotransfer proteins. The phosphoryl group is transferred from the receptor receiver domain to a conserved histidine on the AHP protein. The AHP then translocates into the nucleus to pass the phosphate to response regulators.

**151.** (C) They are transcription factors that activate cytokinin-responsive genes upon phosphorylation B-type Arabidopsis Response Regulators are the final components in the primary cytokinin signaling pathway. These proteins are localized in the nucleus and function as transcription factors. When they receive a phosphoryl group from an AHP protein, they become activated and bind to specific DNA motifs in the promoters of target genes. This triggers the initial transcriptional response to cytokinin, including the activation of division.

**152.** (D) A-type ARRs
To ensure a controlled response, the cytokinin signaling pathway includes a negative feedback loop. When B-type response regulators activate gene expression, they rapidly induce the synthesis of another class of proteins called A-type ARRs. Unlike the B-type, A-type ARRs do not have DNA-binding domains. Instead, they compete for the phosphoryl groups from AHP proteins, effectively dampening the signaling cascade and preventing hyper-responsiveness.

**153.** (B) The receptor is active; it activates CTR1, keeping the signaling pathway OFF
Ethylene signaling is unique because the hormone receptors act as negative regulators of the pathway. In the absence of ethylene, the receptors remain in an active state. They work in conjunction with a protein kinase called CTR1 to actively suppress the downstream signaling components. As long as no hormone is present, the pathway remains in an off state, preventing ethylene-responsive developmental changes from occurring.

**154.** (D) Serine/threonine kinase belonging to the Raf MAPKKK family
CTR1 is a central component of the ethylene signaling pathway that functions as a powerful negative regulator. Structurally, it is a serine/threonine protein kinase that belongs to the Raf family of MAP kinase kinase kinases. When the ethylene receptors are active in the absence of hormone, they bind to and activate CTR1. This kinase then phosphorylates downstream components to prevent the signal from reaching the nucleus.

**155.** (A) I, II, III and IV
Ethylene signal transduction involves a series of inactivation and cleavage events. When ethylene binds, it inactivates the receptors and their partner kinase, CTR1. This leads to the dephosphorylation and subsequent cleavage of the EIN2 protein. The resulting C-terminal fragment of EIN2 then translocates into the nucleus. Here, it stabilizes the EIN3 transcription factor, which had been continuously degraded in the absence of hormone.

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**156.** (C) It is continuously targeted for proteasomal degradation by the F-box proteins $EBF_1$/EBF2
EIN3 is a master transcription factor that coordinates the plant response to ethylene. To prevent an untimely response, EIN3 levels are kept extremely low when the hormone is absent. This is achieved by the action of two F-box proteins, $EBF_1$ and EBF2, which recognize EIN3 and target it for rapid proteasomal degradation. Only when ethylene signaling is initiated does this degradation cease, allowing EIN3 to accumulate.

**157.** (D) Copper ($Cu^+$)
The perception of the gaseous hormone ethylene by its receptors is a remarkable biochemical process. For the ETR1 receptor to effectively bind ethylene, it requires the presence of a specific metal cofactor. This cofactor is a single copper ion in the +1 oxidation state. The copper ion is coordinated within the transmembrane domains of the receptor and is absolutely essential for the high-affinity binding of the hormone.

**158.** (A) Mitochondrial genome
Cytoplasmic male sterility is a widespread trait in the plant kingdom that results in the inability to produce functional pollen. This phenomenon is characterized by maternal inheritance, which indicates that it is not controlled by the nuclear genome. Instead, CMS is primarily specified by genes located within the mitochondrial genome. These genes often interfere with the normal development of the male reproductive organs.

**159.** (C) Recombination events generating novel chimeric open reading frames (ORFs)
The molecular basis of cytoplasmic male sterility is typically linked to large-scale structural changes within the mitochondrial DNA. These changes often result from frequent recombination events between different regions of the genome. Such events can create novel, chimeric open reading frames that are transcribed and translated. The resulting proteins often have disruptive effects on

mitochondrial function, specifically during the energy-demanding stages of pollen development.

**160.** (A) Inner mitochondrial membrane integrity and respiratory electron transport
The chimeric proteins associated with cytoplasmic male sterility are often localized to the inner mitochondrial membrane. Because these proteins are unusual and often contain segments of standard respiratory subunits, they can interfere with the normal assembly or function of the electron transport chain. This disruption leads to a decrease in ATP production or an increase in reactive oxygen species, which specifically harms the pollen-producing tissues.

**161.** (A) They suppress the expression or accumulation of the CMS-associated toxic mitochondrial protein
In agriculture, the use of cytoplasmic male sterility for hybrid seed production requires a way to restore fertility in the final crop. This is achieved using nuclear restorer-of-fertility genes. These genes encode proteins that act within the mitochondria to specifically suppress the expression or accumulation of the toxic CMS-associated protein. By neutralizing the mitochondrial defect, these nuclear genes allow the plant to produce functional pollen again.

**162.** (C) Pentatricopeptide repeat (PPR) proteins
Most nuclear genes that restore fertility to plants with cytoplasmic male sterility belong to a large and diverse family of RNA-binding proteins. Known as pentatricopeptide repeat proteins, these molecules are targeted to organelles like mitochondria and chloroplasts. They have evolved to play critical roles in various aspects of organelle RNA metabolism, making them perfectly suited for suppressing the expression of deleterious mitochondrial transcripts associated with sterility.

**163.** (D) By sequence-specific binding to CMS transcripts, promoting RNA cleavage or preventing translation
Restorer-of-fertility PPR proteins function by directly interacting with the problematic mitochondrial transcripts that cause sterility. These proteins exhibit high sequence specificity, allowing them to bind to the chimeric CMS-associated mRNA. Once bound, they can either facilitate the cleavage and degradation of the transcript or physically block the ribosomes, thereby preventing its translation. Both mechanisms effectively reduce the amount of the toxic protein produced.

**164.** (A) Tandem arrays of 35-amino acid degenerate repeats forming a superhelical structure
PPR proteins are characterized by a unique structural organization that enables their sequence-specific RNA binding. They consist of tandem arrays of a degenerate 35-amino acid motif. These repeats fold into a pair of anti-parallel α-helices and stack together to form a superhelical structure. The specific amino acids at certain positions in each repeat determine the nucleotide that the protein binds, allowing for precise recognition.

**165.** (B) Texas (T)-type CMS
The Texas-type cytoplasmic male sterility system in maize is one of the most famous examples in plant genetics. It is caused by a unique mitochondrial chimeric gene named T-urf13. While this gene confers male sterility, it also makes the plants highly sensitive to a toxin produced by the fungus Bipolaris maydis. This vulnerability led to a major epidemic in the 1970s, highlighting the risks of genetic uniformity.

**166.** (A) Prolamins
Seed storage proteins were historically classified into four major groups based on their solubility in different solvents. This system, known as the Osborne classification, remains widely used today. Within this system, prolamins are defined as the protein fraction that is insoluble in water or salt solutions but highly soluble in aqueous alcohol. These proteins are especially important as the major storage components in many cereal grains.

**167.** (C) Prolamins
In most cereal crops, such as wheat, maize, and barley, a specific class of proteins serves as the primary reservoir for nitrogen and sulfur in the seed. These are the prolamins, which include well-known examples like zein and gliadin. They are synthesized in large quantities in the endosperm and often form specialized structures called protein bodies within the cell, providing essential nutrients for the germinating seedling.

**168.** (D) Globulins
Leguminous plants, including soybean, pea, and common bean, store their protein reserves

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primarily in the form of globulins. These proteins are defined by their solubility in dilute salt solutions. The two major types of legume globulins are the 7S vicilins and the 11S legumins. They are synthesized during seed development and stored in specialized vacuoles, eventually serving as the main source of amino acids during growth.

**169.** (A) They are characteristically rich in proline and glutamine but poor in lysine
Cereal prolamins have a distinctive amino acid composition that influences both their structure and their nutritional value. These proteins are characteristically very rich in the amino acids proline and glutamine. However, from a nutritional perspective, they are often considered incomplete because they contain very low levels of certain essential amino acids, most notably lysine. This deficiency is a major target for crop improvement programs.

**170.** (B) The lumen of the rough endoplasmic reticulum (ER)
The synthesis and storage of prolamins in cereal seeds involve a specialized pathway within the cell. These proteins are translated by ribosomes attached to the rough endoplasmic reticulum and are directly translocated into its lumen. Instead of being transported to other organelles, the prolamins often aggregate and accumulate directly within the ER lumen, forming spherical structures known as protein bodies that remain there until germination.

**171.** (B) Protein storage vacuoles (PSVs)
Unlike the prolamins found in many cereals, the globulin storage proteins in legumes follow a more complex trafficking route. They are initially synthesized on the rough endoplasmic reticulum and then move through the Golgi apparatus. Their final destination is a specialized organelle called the protein storage vacuole. In these vacuoles, the proteins are processed and stored in high concentrations until they are needed by the embryo.

**172.** (C) RY repeat (CATGCATG)
The expression of genes encoding seed storage proteins is restricted to specific stages of seed development. This precise control is achieved through the presence of conserved cis-acting elements in their promoters. One of the most important elements found in many legume globulin genes is the RY repeat, characterized by the sequence CATGCATG. This motif is essential for the high-level, seed-specific transcription of these components.

**173.** (D) ABI3/B3-domain transcription factors
The regulation of seed-specific gene expression involves several classes of transcription factors. The RY repeat, a critical promoter element in many storage protein genes, is specifically recognized by proteins belonging to the B3-domain family. A prominent member of this family is ABI3, also known as VP1 in some species. By binding to the RY repeat, these factors coordinate the massive synthesis of storage proteins.

**174.** (D) Opaque-2 (a bZIP transcription factor)
In maize, the synthesis of zein storage proteins is controlled by a complex network of transcription factors. A central player in this network is the Opaque-2 protein, which belongs to the basic leucine zipper family. Opaque-2 binds specifically to a DNA sequence known as the $O_2$-box within the promoters of certain zein genes. Its activity is essential for the high-level accumulation of these proteins in the endosperm.

**175.** (B) Synthesis of preproprotein → Cleavage of signal peptide in ER → Assembly into trimer → Transport to PSV → Cleavage into acidic/basic chains → Assembly into hexamer
The maturation of 11S legumin-type storage proteins involves several sequential steps. After synthesis as a preproprotein, the signal peptide is removed in the ER. The proprotein then assembles into a trimer before being transported to the protein storage vacuole. Once inside the vacuole, it is cleaved into acidic and basic chains, which finally assemble into a stable hexameric structure, the functional storage form.

**176.** (D) DOF (DNA-binding with one finger) family
The promoters of many cereal prolamin genes contain a conserved DNA motif known as the prolamin box or P-box. This element is specifically recognized by a group of transcription factors called Prolamin Binding Factors. These proteins belong to the DOF family, which is characterized by a unique zinc-finger DNA-binding domain. PBF often works together with other factors, like Opaque-2, to ensure the robust expression of storage proteins.

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activity of these kinases is dephosphorylation. Highly specific protein phosphatases remove the activating phosphate groups from the kinases, effectively returning the signaling pathway to its basal state.

**210.** (B) The Skp1-Cullin-F-box (SCF) complex
Many plant hormone signaling pathways utilize a highly conserved mechanism for regulating gene expression through targeted protein degradation. The Skp1-Cullin-F-box complex is a type of E3 ubiquitin ligase that plays a central role in several of these pathways. In the presence of auxin, jasmonate, or gibberellin, specific F-box proteins identify repressor proteins and target them for ubiquitination. This structural conservation allows for a unified way to control hormone-responsive programs.

**211.** (B) By promoting the $SCF^{COI1}$-mediated degradation of JAZ repressor proteins
The activation of jasmonate-responsive genes follows a mechanism very similar to that of auxin signaling. When the bioactive form of jasmonate, JA-Ile, is present, it acts as a molecular glue that brings together the COI1 receptor and a JAZ repressor protein. The COI1 protein is part of an SCF E3 ubiquitin ligase complex. This interaction leads to the ubiquitination and subsequent degradation of the JAZ repressors, allowing transcription factors to act.

**212.** (B) An insoluble aggregate of storage proteins sequestered in specialized organelles (ER derived or PSV)
During seed development, massive amounts of storage proteins are synthesized and must be efficiently packed within the cell. These proteins often form large, insoluble aggregates known as protein bodies. These structures are typically sequestered within specialized organelles, such as the lumen of the rough endoplasmic reticulum or specialized protein storage vacuoles. This compartmentalization ensures that the proteins are safely stored in high concentrations and are easily accessible during early growth stages.

**213.** (B) Decreased synthesis of α-zeins, leading to a compensatory increase in lysine-rich proteins
The opaque-2 mutation in maize is highly valued because it significantly improves the nutritional profile of the grain. This mutation affects a transcription factor that is normally required for the synthesis of certain zein storage proteins. In the mutant, the production of these zeins is drastically reduced. To maintain the total protein content, the plant increases the synthesis of other proteins that are naturally richer in the essential amino acid lysine.

**214.** (C) Ammonia ($NH_3$ / $NH_4^+$)
In the symbiotic relationship between plants and nitrogen-fixing bacteria, a specialized exchange of nutrients occurs within the nodule. The bacteroids use the nitrogenase enzyme to reduce atmospheric nitrogen to ammonia. This ammonia is then rapidly transported across the symbiosome membrane and into the host plant cytosol. Once in the plant cell, the ammonia is quickly incorporated into organic compounds, primarily amino acids, to be used by the host plant.

**215.** (A) Ureides (Allantoin and Allantoic acid)
Legumes that originate from tropical and subtropical regions, such as soybean and cowpea, have a characteristic method for transporting the nitrogen they fix in their nodules. Instead of using simple amides, these plants convert the assimilated nitrogen into specialized compounds known as ureides, specifically allantoin and allantoic acid. These molecules have a very high nitrogen-to-carbon ratio, making them a highly efficient way to transport fixed nitrogen to shoots.

**216.** (B) Amides (mainly Asparagine and Glutamine)
Leguminous plants from temperate regions, including pea, clover, and alfalfa, utilize a different strategy for nitrogen transport compared to tropical species. In these plants, the ammonia fixed by the symbiotic bacteria is primarily incorporated into the amides asparagine and glutamine within the nodule cells. These amides are then loaded into the xylem and transported throughout the plant to provide the necessary nitrogen for the growth of leaves and other organs.

**217.** (A) Dicarboxylic acids (e.g., malate, succinate)
For nitrogen fixation to occur within root nodules, the host plant must supply the symbiotic bacteria with a continuous source of energy and carbon. The primary compounds provided by the plant are certain four-carbon dicarboxylic acids, such as malate and succinate. These molecules are transported into the bacteroids and metabolized through the tricarboxylic acid cycle to generate

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is synthesized uniquely by an enzyme known as Granule-Bound Starch Synthase. Unlike other starch synthases, GBSS is localized exclusively within the matrix of the starch granule. It works by elongating long glucan chains in a processive manner, ensuring the production of the linear amylose molecule within the growing starch granule.

**186.** (A) Waxy phenotype (amylose-free starch)
Mutations that eliminate the activity of Granule-Bound Starch Synthase have a profound effect on the composition of starch in seeds. Because this enzyme is solely responsible for amylose production, these mutant plants produce starch that consists entirely of amylopectin. This condition is known as the waxy phenotype. Waxy starch has unique culinary and industrial properties, making it highly valuable for various specialized applications in the food industry.

**187.** (D) They form α-1,6 branches via glucan chain transfer
Starch branching enzymes are essential for creating the complex, tree-like structure of the amylopectin molecule. They function by cleaving an existing α-1,4-linked glucan chain and then transferring the released segment to another position on the same or a different chain. By forming a new α-1,6-glycosidic bond at the attachment site, these enzymes create the branch points that allow for the dense packing of glucose.

**188.** (A) They trim improperly placed or excessive branches to promote crystalline packing of amylopectin clusters
The formation of a semicrystalline starch granule requires a highly ordered arrangement of amylopectin branches. Debranching enzymes, such as isoamylase, play a vital role in this process. Although they remove branches, they are necessary for starch synthesis because they trim away improperly placed or excessive branches. This editing process ensures that the remaining branches can form the stable, double-helical structures required for the crystalline regions.

**189.** (B) phytoglycogen
When the activity of certain debranching enzymes is lost, the normal process of starch granule formation is severely disrupted. Instead of producing crystalline amylopectin, the plant accumulates a water-soluble, highly branched glucose polymer known as phytoglycogen. This phenotype is seen in the sugary1 mutant of maize. The failure to trim excessive branches prevents the polymer from crystallizing, leading to a drastically different and less stable form.

**190.** (A) Both A and R are true and R is the correct explanation of A.
The regulation of ADP-glucose pyrophosphorylase by the ratio of 3-phosphoglycerate to inorganic phosphate is a fundamental aspect of plant carbon metabolism. This mechanism ensures that starch synthesis in the leaves is precisely synchronized with the output of the Calvin cycle and the demand for sucrose. By sensing these metabolic intermediates, the enzyme acts as a master switch that partitions carbon toward storage or export.

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**191.** (B) Glucan water dikinase (GWD) and Phosphoglucan water dikinase (PWD)
Starch degradation in leaves at night is a complex process that begins with the modification of the starch granule surface. Crystalline starch is resistant to direct enzymatic attack. To overcome this, enzymes called glucan water dikinase and phosphoglucan water dikinase transfer phosphate groups to glucose residues. This phosphorylation disrupts the crystalline structure, making the glucan chains accessible to various starch-degrading hydrolases and phosphorylases.

**192.** (B) Maltose
B-amylase is a key enzyme involved in the breakdown of starch in many plant tissues. It functions as an exohydrolase that successively removes two-glucose units from the non-reducing ends of the starch chains. The primary end-product of this reaction is maltose. During the night, maltose is the main carbon compound generated from starch degradation in the chloroplast, and it is subsequently exported to the cytosol for metabolism.

**193.** (D) Cytocolic D-enzyme (Disproportionating enzyme 2 / DPE2)
After maltose is exported from the chloroplast into the cytosol, it must be metabolized to provide carbon for sucrose synthesis. This process is not a simple hydrolysis. Instead, a cytosolic enzyme called disproportionating enzyme 2, or DPE2, catalyzes a reaction where one glucose unit from maltose is transferred to a water-soluble glucan,

such as a heteroglycan. The released glucose can then be converted into sucrose precursors.

**194.** (D) SSI
The synthesis of amylopectin involves several different classes of soluble starch synthesases, each with a specialized role in chain elongation. Starch Synthase I is unique because it specifically targets very short glucan chains. It is responsible for elongating the short primers that are created immediately after a new branch is formed by a branching enzyme. This activity is a prerequisite for the further elongation performed by other starch synthesases.

**195.** (A) Chain elongation by soluble SS → Chain cleavage and transfer by SBE → Trimming of disordered branches by DBE → Further elongation and crystallization
The formation of the complex amylopectin cluster involves a coordinated cycle of enzymatic activities. First, soluble starch synthases elongate the existing glucan chains. Next, starch branching enzymes cleave these chains and transfer them to create new branch points. Then, debranching enzymes trim away any disordered or excessive branches. This orchestrated sequence of events allows the branches to align properly and form the crystalline double helices characteristic of starch.

**196.** (B) Leaf mesophyll
Plants synthesize starch in two primary modes: for long-term storage or as a temporary reserve. Transit starch is the form that is synthesized within the chloroplasts of leaf mesophyll cells during the day when photosynthesis is active. This starch is not meant for long-term accumulation but is rapidly degraded during the following night to provide a continuous supply of carbon and energy for the entire plant metabolism.

**197.** (D) The synthesis of ADP-glucose in the cytosol followed by transport into the amyloplast via a specific adenylate translocator
Cereal endosperms have evolved a unique metabolic pathway to maximize the efficiency of starch accumulation. Unlike most other tissues, they possess a major form of ADP-glucose pyrophosphorylase that is located in the cytosol. This necessitates the use of a specialized adenylate translocator in the amyloplast membrane. This transporter moves the ADP-glucose synthesized in the cytosol into the plastid, where it is used directly as a substrate.

**198.** (A) Monomerization via reduction of intermolecular disulfide bonds mediated by thioredoxin
The activity of ADP-glucose pyrophosphorylase is regulated by the light status of the plant through a redox-sensitive mechanism. In the light, the reduction of an intermolecular disulfide bond, mediated by the thioredoxin system, causes the enzyme complex to become more active. This post-translational modification increases the enzyme affinity for its activator and decreases its sensitivity to its inhibitor, thereby stimulating starch synthesis during the day.

**199.** (A) Mediation by light-responsive transcription factors such as HY5 binding to light-responsive elements (LREs)
The expression of nuclear genes encoding photosynthetic components, such as the small subunit of RuBisCO, is tightly regulated by light. This process is mediated by specific transcription factors that respond to signals from various photoreceptors. HY5 is a key transcription factor that accumulates in the light and binds directly to light-responsive elements in target promoters. This binding initiates the high-level transcription required for the photosynthetic machinery.

**200.** (B) Saturation or restriction of the cytochrome c oxidase pathway leading to high mitochondrial ROS
The alternative oxidase is a mitochondrial protein that provides a pathway for electrons to bypass the cytochrome c oxidase complex. Its expression is often induced as a retrograde signaling response when the normal respiratory chain is compromised or saturated. Such conditions lead to an increase in the production of mitochondrial reactive oxygen species. These ROS act as signals that trigger the nucleus to upregulate the AOX gene.

**201.** (A) Azelaic acid, Pipecolic acid, and Salicylic acid derivatives
Systemic acquired resistance is a mechanism by which an entire plant becomes more resistant to future attacks following a localized infection. This process requires the movement of long-distance signals through the vascular system. Several small molecules have been identified as mobile signals, including pipecolic acid,

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azelaic acid, and certain derivatives of salicylic acid. Together, these molecules prepare distant tissues to respond more rapidly and effectively to pathogens.

**202.** (A) K. pneumoniae organizes them in a clustered ~24 kb region; Rhizobium species typically have them scattered, often on large symbiotic plasmids
The genetic organization of nitrogen fixation genes varies significantly between different diazotrophs. In the free-living bacterium Klebsiella pneumoniae, the nif genes are organized into a large, tightly clustered region of about twenty-four kilobases. In contrast, in symbiotic bacteria like Rhizobium species, these genes are often more widely scattered throughout the genome. Many Rhizobia carry their essential symbiotic and nitrogen fixation genes on large symbiotic plasmids.

**203.** (B) They induce terminal bacteroid differentiation, cell enlargement, and block bacterial cell division
In certain legumes, such as Medicago truncatula, the host plant produces a large family of specialized peptides called Nodule-specific Cysteine-Rich peptides. These peptides are delivered to the symbiotic bacteria within the symbiosome. They play a critical role in inducing terminal differentiation of the bacteria into large, non-dividing bacteroids. This morphological change is associated with an increase in genomic DNA and is essential for establishing efficient nitrogen-fixing symbiosis.

**204.** (B) The intracellular kinase domain of NFR1
The perception of Nod factors by the NFR1 and NFR5 receptor-like kinases initiates a signaling cascade through their intracellular domains. While NFR5 is a pseudokinase that lacks catalytic activity, NFR1 is a functional kinase. Upon the successful binding of the bacterial signal to the extracellular LysM domains, the kinase domain of NFR1 undergoes phosphorylation. This activation of the NFR1 kinase is a critical early event in signal transmission.

**205.** (C) Zinc and Cadmium
Heavy metal ATPases are essential for the movement and detoxification of metal ions in plants. In Arabidopsis, the HMA4 transporter is a key protein involved in the systemic distribution of certain metals. Localized to the plasma membrane of the root pericycle, HMA4 functions by loading zinc and cadmium into the xylem. This activity facilitates the transport of these metals from roots to shoots, playing a vital role in metal tolerance.

NODIA

**206.** (C) Plasma membrane-localized NADPH oxidases (RBOHs)
The oxidative burst is a fundamental part of the plant immune response to pathogens. The primary source of the reactive oxygen species produced during this burst is a family of plasma membrane-localized enzymes called Respiratory Burst Oxidase Homologs. These are NADPH oxidases that use cytosolic NADPH to produce superoxide in the apoplast. Their activity is rapidly triggered by various signaling events, including calcium influx and phosphorylation during PAMP-triggered immunity.

**207.** (A) Fluidity decreases (rigidification), likely perceived by mechanosensitive calcium channels
When plants are exposed to low temperatures, the physical state of their cellular membranes changes significantly. Cold temperatures cause the lipid bilayer to become more rigid, leading to a decrease in membrane fluidity. This rigidification is thought to be a primary sensing event that is perceived by the cell, possibly through the activation of mechanosensitive calcium channels. The resulting calcium influx acts as a signal to initiate cold acclimation.

**208.** (A) Increased proportion of unsaturated fatty acids
To maintain the functionality of cellular processes during cold acclimation, plants must adjust the composition of their membranes. The primary strategy to compensate for cold-induced rigidification is to increase the proportion of unsaturated fatty acids in the membrane lipids. By introducing more double bonds into the fatty acid chains, the plant lowers the melting point of the lipids, thereby maintaining the necessary fluidity of the membranes at low temperatures.

**209.** (A) Dephosphorylation by highly specific protein phosphatases (e.g., MAPK phosphatases, PP2Cs)
Both MAP kinase and calcium-dependent protein kinase pathways are essential for mounting rapid responses to stress. However, to prevent excessive signaling that could be harmful to the cell, these pathways must be carefully controlled. The most common regulatory mechanism for limiting the

**177.** (B) A vacuolar processing enzyme (VPE), which is a legumain-type cysteine protease
Once storage proteins arrive at the protein storage vacuole, they often require proteolytic processing to reach their final, stable form. This maturation is catalyzed by a specific class of cysteine proteases known as vacuolar processing enzymes or legumains. These enzymes are highly specific and cleave the storage proproteins at certain asparagine residues, which is a critical step for the proper assembly and packing of the protein stores.

**178.** (C) Formation of numerous intra- and inter-chain disulfide bonds
Seed storage proteins must be highly stable to remain intact throughout seed dormancy. This stability is largely achieved through structural modifications that occur during their folding in the rough endoplasmic reticulum. Specifically, the formation of numerous intramolecular and intermolecular disulfide bonds is essential. These covalent bonds between cysteine residues help to lock the proteins into their correct shapes and facilitate the formation of large, insoluble protein aggregates.

**179.** (A) Amylose and amylopectin
Starch is the primary storage carbohydrate in plants and is deposited as insoluble granules within specialized organelles called plastids. Chemically, it consists of two distinct types of glucose polymers. Amylose is a relatively small, primarily linear molecule, while amylopectin is a much larger and highly branched polymer. The specific ratio and arrangement of these two components determine the physical and chemical properties of the starch granule.

**180.** (B) Amylose is essentially linear with α-1,4 linkages; amylopectin is highly branched with α-1,6 linkages
The two glucan polymers that make up starch differ fundamentally in their architecture. Amylose consists of long, essentially linear chains of glucose units connected by α-1,4-glycosidic bonds. In contrast, amylopectin is a highly branched molecule. While it also contains α-1,4-linked chains, it features numerous branch points created by α-1,6-glycosidic bonds. This branching structure is essential for the formation of the semicrystalline matrix of the starch granule.

**181.** (C) The synthesis of ADP-glucose by ADP-glucose pyrophosphorylase (AGPase)
The biosynthesis of starch is a highly regulated metabolic pathway in plants. The primary committed step is the production of the activated sugar-nucleotide precursor, ADP-glucose. This reaction is catalyzed by the enzyme ADP-glucose pyrophosphorylase, which uses glucose-1-phosphate and ATP as substrates. Because it controls the flow of carbon into starch synthesis, this enzyme is a major site of regulation by both allosteric effectors and light signals.

**182.** (D) 3-Phosphoglycerate (3-PGA)
The activity of ADP-glucose pyrophosphorylase is finely tuned to the rate of photosynthesis. In leaf tissues, the primary allosteric activator of this enzyme is 3-phosphoglycerate, which is the first product of carbon fixation in the Calvin cycle. When 3-phosphoglycerate levels are high, it indicates an abundance of fixed carbon and energy, signaling the enzyme to increase the production of ADP-glucose for starch synthesis.

**183.** (C) Inorganic phosphate (Pi)
To coordinate starch production with the metabolic needs of the cell, ADP-glucose pyrophosphorylase is subject to potent allosteric inhibition. Inorganic phosphate serves as the primary inhibitor of this enzyme. High levels of phosphate indicate that the cell energy and carbon resources are being used elsewhere, leading to the suppression of starch synthesis. The balance between 3-phosphoglycerate and phosphate is the critical factor in controlling activity.

**184.** (D) A large proportion of AGPase activity is localized in the cytosol, requiring ADP-glucose transport into the amyloplast
In non-photosynthetic tissues like the developing endosperm of cereals, the organization of starch synthesis differs from that of leaves. While starch is synthesized in the amyloplast, a significant portion of the ADP-glucose pyrophosphorylase activity is localized within the cytosol. This arrangement requires a specialized transport protein to move the ADP-glucose synthesized in the cytosol across the amyloplast membrane, where it can be used for polymer synthesis.

**185.** (B) Granule-Bound Starch Synthase (GBSS)
The synthesis of the two types of starch polymers is handled by different classes of enzymes. Amylose

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the ATP and reducing power needed to drive the energy-intensive nitrogenase reaction.

**218.** (B) The P-cluster ([8Fe-7S])
The molybdenum-iron protein of the nitrogenase complex contains two essential metal clusters that facilitate the reduction of nitrogen. In addition to the FeMoco active site, the protein houses a second complex known as the P-cluster. This cluster consists of eight iron and seven sulfur atoms. Its primary role is to serve as an intermediate electron carrier, accepting electrons from the iron protein and passing them on to the FeMoco.

**219.** (A) Malate
C4 photosynthesis involves a specialized shuttle that concentrates carbon dioxide around the RuBisCO enzyme. In NADP-malic enzyme type plants, such as maize, the initial product of carbon fixation is converted into malate in the mesophyll cells. This four-carbon acid is then transported through plasmodesmata into the neighboring bundle sheath cells. Once there, the malate is decarboxylated to release carbon dioxide, which is then used by RuBisCO.

**220.** (A) Pyruvate
In the C4 photosynthetic pathway, the carbon shuttle must be completed to allow for continuous fixation. After malate is transported to the bundle sheath cells and decarboxylated to release carbon dioxide, a three-carbon compound called pyruvate is produced. This pyruvate is then transported back from the bundle sheath cells to the mesophyll cells. In the mesophyll, it is used to regenerate the initial carbon acceptor, phosphoenolpyruvate, completing the cycle.

**221.** (A) By concentrating $CO_2$ at the site of RuBisCO in the bundle sheath, strongly favoring carboxylase over oxygenase activity
C4 plants utilize a unique leaf structure called Kranz anatomy to significantly reduce the rate of photorespiration. By separating the initial carbon fixation and the Calvin cycle into two different cell types, the plant creates a $CO_2$-concentrating mechanism. This ensures that the RuBisCO enzyme in the bundle sheath cells is surrounded by a very high concentration of carbon dioxide. This environment strongly favors the carboxylase activity of RuBisCO over oxygenase activity.

**222.** (A) Because the first unique product of the oxygenase reaction is a 2-carbon compound (2-phosphoglycolate)
The photorespiratory pathway is commonly referred to as the C2 cycle because of the nature of the first unique intermediate formed in the process. When RuBisCO reacts with oxygen instead of carbon dioxide, it produces a two-carbon molecule called 2-phosphoglycolate. The entire photorespiratory pathway is dedicated to recycling this two-carbon compound back into the three-carbon intermediates of the Calvin cycle, minimizing the loss of carbon and energy that would occur.

**223.** (B) Consumption of 2 ATP and 2 reducing equivalents (NAD(P)H or ferredoxin) per oxygenation event
Photorespiration is an energy-intensive process that places a significant metabolic burden on the plant. For every oxygenation event by RuBisCO, the plant must expend a considerable amount of energy to recover the lost carbon. This process minimally consumes two molecules of ATP and two reducing equivalents, such as NADH or reduced ferredoxin. This high energy cost explains why C4 plants have evolved specialized mechanisms to suppress photorespiration and increase efficiency.

**224.** (A) Mitochondrial malate dehydrogenase (coupled with substrate shuttles)
During photorespiration, the conversion of glycine to serine in the mitochondria generates a molecule of NADH. For the pathway to continue, this NADH must be rapidly reoxidized. This is primarily achieved through the action of mitochondrial malate dehydrogenase in conjunction with various substrate shuttles, such as the malate-oxaloacetate shuttle. This mechanism allows the reducing equivalents to be transferred out of the mitochondria or used in other metabolic processes for redox balance.

**225.** (B) Efficient reassimilation by the plastidic glutamine synthetase (GS2) and Fd-GOGAT
The large amount of ammonia released during the glycine decarboxylation step of photorespiration must be efficiently recycled to prevent toxicity. Plants have evolved a powerful reassimilation system for this purpose. The ammonia is transported from the mitochondria to the chloroplast, where it is incorporated back into organic form by the enzymes glutamine

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2013

**37.** Somaclonal variation refers to the genetic and epigenetic variations observed in plants regenerated from:
(A) Any form of somatic cell or tissue culture.
(B) Cultures derived from meristem tips only.
(C) Zygotic embryos germinated in petri dishes.
(D) Seeds directly germinated under in vivo conditions.

2006

**38.** Which of the following is an epigenetic mechanism responsible for somaclonal variation?
(A) Recurrent point mutations.
(B) Major chromosomal translocations.
(C) Altered DNA methylation patterns.
(D) Random transposon activation.

2016

**39.** In vitro selection for abiotic stress tolerance involves exposing plant cell cultures to selective agents. To screen for salinity tolerance, which agent is appropriately added to the medium?
(A) Sodium chloride (NaCl)
(B) Polyethylene glycol (PEG)
(C) Fusaric acid
(D) Streptomycin

2022

**40.** Consider the following statements concerning in vitro mutagenesis:
I. EMS (Ethyl methanesulfonate) is a highly effective chemical mutagen.
II. LD50 represents the dose of a mutagen that kills 50% of the explants/cells.
III. Gamma rays cause physical mutations primarily through single-strand breaks and translocations.
IV. In vitro mutagenesis restricts mutation screening strictly to the $M1$ generation.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I and IV

2012

**41.** Secondary metabolite production in plant cell cultures is often optimized by the addition of elicitors. Which of the following is classified as an abiotic elicitor?
(A) Ultraviolet (UV) radiation
(B) Chitin
(C) Glucans from fungal cell walls
(D) Yeast extract

NODIA

2018

**42.** Hairy root cultures are renowned for their rapid growth and high secondary metabolite production. They are induced by infecting plant tissues with:
(A) Agrobacterium tumefaciens
(B) Agrobacterium rhizogenes
(C) Bacillus thuringiensis
(D) Pseudomonas syringae

2003

**43.** The genetic basis for the induction of hairy roots lies in the integration and expression of which specific genes from the Ri plasmid?
(A) vir genes
(B) nptII genes
(C) cry genes
(D) rol genes (rol A, B, C, D)

2014

**44.** Which bioreactor design is specifically preferred for fragile plant cell suspension cultures because it provides adequate mixing with minimal hydrodynamic shear stress?
(A) Stirred tank bioreactor with high-speed Rushton turbines
(B) Fixed bed bioreactor
(C) Membrane bioreactor
(D) Airlift bioreactor

2024

**45.** Plant cell immobilization offers advantages for continuous secondary metabolite production. Which matrix is most commonly used for entrapping plant cells?
(A) Agarose gel
(B) Calcium alginate beads
(C) Polyvinyl chloride (PVC)
(D) Silicon carbide

2006

**46.** Biotransformation using plant cell cultures refers to the process where:
(A) Cells are genetically transformed to produce novel proteins.
(B) Plant cells convert inorganic nitrogen into organic acids.
(C) Cells modify an exogenous precursor into a valuable product.
(D) The physical state changes from solid callus to suspension.

2019

**47.** Cryopreservation of plant germplasm involves the storage of biological materials at ultra-low temperatures, typically using:

chlorophyll absorbs red light but reflects far-red light, the light within a canopy is enriched in far-red wavelengths. This results in a low red to far-red ratio. This specific environmental cue is detected by phytochromes and triggers the shade avoidance response, which includes accelerated stem elongation for light.

**234.** (B) PIF4 and PIF5
During the shade avoidance response, the reduction in active phytochrome Pfr levels has a direct impact on several transcription factors. Under these conditions, the levels of Phytochrome Interacting Factors 4 and 5 increase in the nucleus. Because there is less active phytochrome to promote their degradation, these PIFs accumulate and activate the expression of genes involved in auxin biosynthesis and cell wall remodeling. This molecular shift leads to the characteristic elongation.

**235.** (C) Casein Kinase 2 (CK2) or directly by Phytochrome itself functioning as a weak kinase
The light-induced degradation of Phytochrome Interacting Factors is a complex process that often begins with their phosphorylation. Several kinases have been implicated in this event, including Casein Kinase 2 and certain MAP kinases. In some cases, the phytochrome molecule itself may possess weak kinase activity and directly phosphorylate the PIFs upon binding. This phosphorylation serves as a critical signal that targets the transcription factors for ubiquitination and subsequent destruction.

**236.** (C) They are essential co-factors that enhance the substrate-targeting and ubiquitination activity of COP1
The COP1-SPA complex is a major repressor of light signaling that functions as an E3 ubiquitin ligase. Within this complex, the SPA proteins play a vital regulatory role. They do not possess the catalytic RING-finger themselves, but they are essential co-factors that physically interact with COP1. The presence of SPA proteins significantly enhances the ability of COP1 to identify its substrates, such as HY5, and to catalyze the transfer of ubiquitin.

**237.** (A) Retrograde signals coordinate nuclear gene expression with plastid developmental status and environmental stresses
Retrograde signaling is a fundamental communication process that allows chloroplasts to relay information about their functional and developmental state to the nucleus. This system ensures that the expression of nuclear-encoded genes for photosynthetic proteins is perfectly coordinated with the needs of the plastid. By responding to environmental stresses and internal metabolic cues from the chloroplast, the nucleus can adjust overall development and stress response to maintain cellular homeostasis and optimize growth.

**238.** (B) Phytoene desaturase in the carotenoid biosynthesis pathway, leading to photo-oxidative bleaching
Norflurazon is a widely used tool for studying chloroplast-to-nucleus retrograde signaling. It functions by specifically inhibiting the enzyme phytoene desaturase, which is a key component of the carotenoid biosynthetic pathway. When plants are treated with this inhibitor, they become deficient in carotenoids and lose their ability to protect themselves from photooxidative damage. The resulting bleaching of the plastids generates a powerful stress signal that causes the downregulation of many nuclear genes.

**239.** (A) Pentatricopeptide repeat (PPR) proteins with a Small MutS-Related (SMR) domain
GUN1 is a central and highly conserved protein in the chloroplast that integrates multiple retrograde signaling pathways. Structurally, GUN1 belongs to the pentatricopeptide repeat family of proteins and contains a specialized Small MutS-Related domain at its C-terminus. Although its precise molecular function is still being elucidated, GUN1 is thought to act as a hub that senses various plastid stresses, such as impaired protein synthesis or altered tetrapyrrole metabolism, and initiates the signal.

**240.** (C) HSP100 (e.g., ClpB)
During periods of extreme heat stress, cellular proteins can lose their correct shape and form large, rigid aggregates. While many chaperones prevent this process, the HSP100 family, also known as the ClpB family, has the unique ability to reverse it. These proteins use energy from ATP hydrolysis to physically untangle and pull individual polypeptide chains out of the clumped aggregates. This disaggregation activity is essential for allowing the proteins to be refolded.

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(D) Torpedo, Heart, Globular, Cotyledonary

2021

**19.** Artificial seeds are produced by encapsulating somatic embryos. Which chemical combination is most widely used for this encapsulation process?
(A) Sodium alginate and calcium chloride
(B) Potassium alginate and sodium chloride
(C) Polyacrylamide and copper sulfate
(D) Agarose and ammonium nitrate

2010

**20.** In the context of androgenesis, anther culture differs from microspore culture in that anther culture:
(A) Involves the culture of isolated, wall-free pollen grains.
(B) Eliminates the influence of somatic anther tissues like the tapetum.
(C) Always results in triploid plant production.
(D) Cultivates the intact anther containing microspores.

2023

**21.** Which developmental pathway of pollen embryogenesis (known as Pathway I) involves the equal division of the microspore?
(A) The vegetative cell divides to form the haploid embryo.
(B) The microspore divides symmetrically into identical cells.
(C) The generative cell divides to form the haploid embryo.
(D) Both vegetative and generative cells fuse before dividing.

2023

**22.** Colchicine is widely used in haploid breeding to produce doubled haploids. How does colchicine achieve chromosome doubling?
(A) By stimulating rapid DNA replication during the G1 phase
(B) By inducing direct fusion of two microspores
(C) By preventing the formation of spindle fibers during mitosis
(D) By inhibiting cytokinesis without affecting karyokinesis

2010

**23.** Gynogenesis is the process of generating haploid plants via the in vitro culture of:
(A) Anthers or pollen grains
(B) Immature zygotic embryos
(C) Endosperm tissues
(D) Unfertilized ovaries or ovules

NODIA

2018

**24.** Which of the following is a primary application of gynogenesis over androgenesis?
(A) Production of haploids in male-sterile crop species.
(B) Rapid induction and production of triploid plants.
(C) Direct intracellular transfer of active chloroplasts.
(D) Complete eradication of systemic viral pathogens.

2025

**25.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Embryo rescue is an effective technique to overcome post-zygotic incompatibility in interspecific hybridization.
Reason (R): The technique involves the in vitro culture of immature hybrid embryos before they abort due to endosperm failure.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2006

**26.** Which of the following barriers to distant hybridization is NOT mitigated by embryo rescue?
(A) Premature degeneration of the endosperm tissue.
(B) Failure of the pollen tube to reach the stigma.
(C) Spontaneous abortion of the embryo post-zygote.
(D) Nutrient starvation of the developing hybrid embryo.

2023

**27.** Endosperm culture is specifically utilized in plant tissue culture for the direct production of:
(A) Triploid plants
(B) Haploid plants
(C) Diploid true-to-type plants
(D) Hexaploid plants

2005

**28.** Why is triploid production via endosperm culture particularly valuable in horticultural fruit breeding?
(A) It induces high seed set for greater propagation.

as an essential cofactor for this process. PTST contains a carbohydrate-binding module that allows it to bind to the starch granule and specifically deliver GBSS to its site of action. Without PTST, GBSS cannot efficiently accumulate in the starch granule.

**249.** (B) Amylose is synthesized inside the growing amylopectin matrix; therefore, a substantial amylopectin matrix must form first before GBSS can act optimally

In the development of many cereal seeds, the accumulation of amylose is often observed to lag behind the synthesis of amylopectin. This phenomenon is largely explained by the structural relationship between the two glucan polymers. Amylose is synthesized within the pre-existing matrix of the larger, branched amylopectin molecules. Therefore, a substantial amylopectin framework must first be established within the growing starch granule before the Granule-Bound Starch Synthase can effectively begin to deposit amylose.

**250.** (B) Through redox-regulation and the physical separation of degradative enzymes alongside allosteric controls

Plants must precisely coordinate the synthesis and degradation of starch to ensure a steady supply of energy. This is achieved through a combination of several regulatory mechanisms. During the day, the starch-degrading enzymes are kept inactive through redox-based regulation and allosteric inhibition by photosynthetic intermediates. Additionally, the physical separation of certain enzymes and the requirement for specific modifications like phosphorylation ensure that the starch granule remains stable during the light and is only degraded.

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**241.** (A) Sequence-specific base pairing of the siRNA to the viral mRNA, guiding AGO to cleave the target
The core mechanism of plant antiviral defense through RNA interference relies on the precision of small interfering RNAs. These small RNAs are loaded into Argonaute proteins to form the RNA-induced silencing complex. The siRNA then acts as a sequence-specific guide, using base pairing to identify complementary viral mRNA molecules. Once the target is found, the Argonaute protein uses its catalytic activity to cleave the viral RNA, effectively silencing the virus and infection.

**242.** (C) Salicylic acid (SA)
Systemic acquired resistance is characterized by the widespread induction of defense genes and increased immunity throughout the plant. A hallmark of this process is the massive accumulation of the phytohormone salicylic acid in both the primarily infected and the distant, uninfected tissues. Salicylic acid serves as a central signal that triggers the expression of pathogenesis-related genes and primes the entire plant to react more rapidly and aggressively to any future pathogens.

**243.** (A) SA alters cellular redox state and binds to NPR receptors, causing NPR1 oligomers to dissociate into active monomers that enter the nucleus
NPR1 is the master regulator of the plant response to salicylic acid during systemic acquired resistance. In the absence of a signal, NPR1 exists as large, inactive oligomers in the cytoplasm. When salicylic acid levels rise, the cellular redox state changes, and the hormone binds to its receptors. This triggers the dissociation of the NPR1 oligomers into active monomers. These monomers then translocate into the nucleus, where they can interact with transcription factors.

**244.** (B) WRKY and TGA (bZIP) transcription factors
Once NPR1 enters the nucleus following salicylic acid activation, it does not bind directly to DNA. Instead, it functions as a transcriptional co-activator by physically interacting with several families of transcription factors. Specifically, NPR1 binds to members of the TGA family of basic leucine zipper proteins and various WRKY transcription factors. Through these interactions, NPR1 enhances the ability of these factors to bind to the promoters of pathogenesis-related genes and stimulate expression.

**245.** (B) A hexameric complex composed of six acidic-basic heterodimers held by disulfide bonds
11S globulins, such as glycinin from soybean, are major seed storage proteins with a complex quaternary structure. Each functional protein unit is a large hexamer composed of six subunits. Each subunit is itself a heterodimer consisting of an acidic polypeptide and a basic polypeptide, which are linked together by a single disulfide bond. This highly organized arrangement is achieved through several processing steps in the ER and the protein storage vacuole, resulting in stable storage.

**246.** (B) They are encoded by large multi-gene families that arose via extensive tandem duplication and mutation
Cereal prolamins exhibit a high degree of polymorphism, which makes their genetic analysis particularly challenging. This diversity is not due to complex RNA processing but rather to the organization of their genes. Prolamins are encoded by large multi-gene families that have arisen through extensive processes of tandem duplication and subsequent mutation. This evolutionary history has resulted in many closely related gene copies that produce a wide array of similar but distinct protein isoforms.

NODIA

**247.** (A) ABI3
Abscisic acid is the primary hormone responsible for inducing and maintaining seed dormancy, preventing the seeds from germinating prematurely. This developmental process is mediated by several seed-specific transcription factors. ABI3 is a master regulator of seed maturation that is strongly upregulated by ABA. By binding to specific promoter elements, ABI3 activates a suite of genes that establish dormancy and ensure that the seed remains viable and quiescent until environmental conditions favor growth.

**248.** (C) Proteolipid / Protein Targeting to Starch (PTST)
The synthesis of amylose by Granule-Bound Starch Synthase requires the enzyme to be properly localized and active within the starch granule. A recently discovered protein called Protein Targeting to Starch 1, or PTST, acts

2023

**9.** Cellular totipotency in plant tissue culture is defined as the inherent ability of a plant cell to:
(A) Undergo rapid meiosis and form haploid gametes in vitro.
(B) Regenerate into a complete whole plant under appropriate stimuli.
(C) Differentiate into a highly specialized dead structural cell.
(D) Secrete excessive secondary metabolites when subjected to stress.

2010

**10.** According to the Skoog-Miller model, which hormonal ratio specifically induces shoot organogenesis from callus cultures?
(A) High auxin to low cytokinin ratio
(B) Low auxin to high cytokinin ratio
(C) Equal concentrations of auxin and cytokinin
(D) High gibberellin to low abscisic acid ratio

2016

**11.** Which process is characterized by the conversion of mature, differentiated cells back to a meristematic state to form a callus?
(A) Dedifferentiation
(B) Redifferentiation
(C) Habituation
(D) Somaclonal variation

2010

**12.** During micropropagation, Stage II is primarily concerned with which of the following objectives?
(A) Acclimatization of plantlets to ex vitro conditions
(B) Initial establishment of the aseptic culture
(C) Rapid multiplication of shoots
(D) Induction of adventitious roots

2022

**13.** Which technique is most effective for the elimination of systemic viruses from a deeply infected mother plant?
(A) Mature embryo rescue and culture of zygotic embryos.
(B) Haploid production through anther or pollen culture.
(C) Transformation mediated by Agrobacterium tumefaciens.
(D) Meristem tip culture combined with heat treatment.

2021

**14.** Which statement represents the best description of chemotherapy in plant tissue culture?
(A) Treating explants with physical mutagens to induce variation.
(B) Exposing plantlets to heat to kill bacterial endophytes.
(C) Supplementing the medium with antiviral agents like ribavirin.
(D) Using colchicine to induce polyploidy in micropropagated plants.

2021

**15.** In the growth kinetics of plant cell suspension cultures, the phase characterized by the maximum rate of cell division is the:
(A) Lag phase
(B) Log (exponential) phase
(C) Stationary phase
(D) Decline phase

2001

**16.** Match the following terms related to cell culture (List I) with their descriptions (List II).
List I: (Cell Culture Term)
P. Bergmann's cell plating
Q. Micro-chamber technique
R. Lag phase
S. Decline phase
List II: (Description)
1. Adaptation of cells to fresh medium
2. Observation of single cell division under a microscope
3. Cell death due to nutrient depletion
4. Embedding cells in a thin layer of agar medium
(A) P-2, Q-4, R-3, S-1
(B) P-4, Q-1, R-2, S-3
(C) P-1, Q-4, R-2, S-3
(D) P-4, Q-2, R-1, S-3

2019

**17.** The primary difference between direct and indirect somatic embryogenesis is that indirect somatic embryogenesis involves:
(A) An intervening callus phase prior to embryo formation
(B) Formation of embryos directly from explant epidermal cells
(C) Formation of zygotic embryos in vitro
(D) Obligate use of Agrobacterium tumefaciens

2025

**18.** What is the correct sequential order of developmental stages during somatic embryogenesis in dicots?
(A) Globular, Torpedo, Heart, Cotyledonary
(B) Heart, Globular, Cotyledonary, Torpedo
(C) Globular, Heart, Torpedo, Cotyledonary

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# CHAPTER 10
# Tissue Culture

NODIA

2024

**1.** Which of the following nitrogen sources is predominantly present in the B5 medium developed by Gamborg?
(A) High concentration of potassium nitrate
(B) High concentration of ammonium nitrate
(C) Equal proportions of ammonium and nitrate ions
(D) Ammonium chloride as the sole nitrogen source

2017

**2.** In plant tissue culture, myo-inositol is frequently added to the culture media. What is its primary physiological role?
(A) It acts as the primary carbon source replacing sucrose.
(B) It serves as a volatile chelating agent for iron.
(C) It functions as a structural component of the cell wall and aids in membrane biosynthesis.
(D) It irreversibly inhibits ethylene biosynthesis in sealed vessels.

2025

**3.** White's medium was originally developed for the continuous culture of which specific plant part?
(A) Excised tomato roots
(B) Apical meristems
(C) Protoplasts
(D) Immature embryos

2024

**4.** Identify the incorrect statement regarding the macronutrients in Murashige and Skoog (MS) medium.
(A) MS medium contains high levels of nitrate and ammonium salts.
(B) Calcium chloride is utilized to provide essential calcium ions.
(C) Potassium serves as a major osmoticum and enzyme activator.
(D) The total ionic concentration of MS medium is significantly lower than that of White's medium.

2022

**5.** During the sterilization of plant tissue culture media, thermolabile compounds must be sterilized separately. Which of the following is typically sterilized using membrane filtration?
(A) Agar
(B) Gibberellic acid (GA3)
(C) Sucrose
(D) Potassium dihydrogen phosphate

2011

**6.** What is the standard physical parameter for sterilizing agar-gelled tissue culture media using an autoclave?
(A) $100^{\circ}C$ at $10psi$ for 30 minutes
(B) $110^{\circ}C$ at $5psi$ for 45 minutes
(C) $121^{\circ}C$ at $15psi$ for 15-20 minutes
(D) $135^{\circ}C$ at $20psi$ for 5 minutes

2010

**7.** A researcher is using mercuric chloride ($HgCl_2$) as a surface sterilant for woody plant explants. Which of the following represents a major disadvantage of this chemical?
(A) It acts primarily as a fungistatic agent with limited bactericidal efficacy.
(B) It evaporates rapidly during application, reducing its effective surface exposure.
(C) It specifically targets and denatures endogenous plant auxins on contact.
(D) It is highly toxic and requires thorough washing to prevent injury.

2008

**8.** Consider the following statements regarding chemical sterilization in tissue culture:
I. Sodium hypochlorite relies on the release of chlorine for its microbicidal action.
II. Ethanol (70%) requires an exposure time of 30 minutes for effective explant sterilization.
III. Tween-20 is often added to sterilants to reduce surface tension.
Which of the above statement(s) is/are correct?
(A) I and III
(B) II
(C) I and II
(D) III

(B) It results in seedless fruits due to meiotic sterility.
(C) It enhances photosynthesis by modifying chloroplast structure.
(D) It makes plants resistant to all known viral pathogens.

2004

**29.** Consider the following statements regarding protoplast isolation:
I. Cellulases are used to degrade the pectin middle lamella.
II. Macerozyme primarily degrades the hemicellulose and pectin components.
III. Osmotic stabilizers are omitted during enzymatic digestion to speed up isolation.
IV. Plasmolysis of cells occurs prior to cell wall degradation when placed in the enzyme mixture.
Which of the above statement(s) is/are incorrect?
(A) I and III
(B) II, III and IV
(C) I, II and IV
(D) III and IV

2016

**30.** During protoplast isolation, mannitol and sorbitol are routinely added to the enzyme solution. What is their primary function?
(A) To provide a rapidly metabolizable carbon and energy source.
(B) To act as osmotic stabilizers to prevent protoplast lysis.
(C) To buffer the pH of the enzyme mixture to a neutral 7.5.
(D) To chelate calcium ions required for cellular membrane stability.

2010

**31.** Fluorescein diacetate (FDA) is used for testing protoplast viability. Viable protoplasts fluoresce green under UV light because:
(A) FDA is converted to fluorescein by intracellular esterases.
(B) FDA binds directly to the cellulose in the cell wall.
(C) FDA selectively stains the nuclear DNA of living cells.
(D) FDA accumulates in the central vacuole via active transport.

2007

**32.** Which of the following chemical fusogens is most widely utilized for inducing protoplast fusion due to its high efficiency and low toxicity?
(A) Sodium dodecyl sulfate (SDS)
(B) Polyethylene glycol (PEG)
(C) Mercuric chloride
(D) Colchicine

2017

**33.** Arrange the standard steps of protoplast electrofusion in the correct sequence.
I. Application of a short, high-voltage direct current (DC) pulse
II. Membrane breakdown and subsequent fusion of adjacent protoplasts
III. Application of a low-voltage alternating current (AC) field
IV. Pearl chain formation (alignment of protoplasts)
(A) I, II, III, IV
(B) III, I, IV, II
(C) IV, III, I, II
(D) III, IV, I, II

NODIA

2015

**34.** What distinguishes a cybrid from a somatic hybrid?
(A) A cybrid possesses nuclear genomes of both parents but no cytoplasm.
(B) A cybrid is formed exclusively through generative cell fusion in vitro.
(C) It has one parental nucleus and the other parent's cytoplasm.
(D) A cybrid is a strictly haploid organism derived from somatic tissue.

2017

**35.** The donor-recipient method for organelle transfer in cybridization typically involves:
(A) Gamma irradiation of the donor protoplast to inactivate its nucleus.
(B) Heavy metal treatment of the recipient protoplast.
(C) Complete removal of chloroplasts from both parents.
(D) Spontaneous fusion without any chemical or electrical stimulus.

2013

**36.** Cytoplasmic male sterility (CMS) in crops is an important trait that is transferred via cybridization because the genes for CMS are located in the:
(A) Nuclear genome
(B) Endoplasmic reticulum
(C) Golgi apparatus
(D) Mitochondrial genome

synthetase 2 and ferredoxin-dependent glutamate synthase. This system ensures that the nitrogen is preserved and reused for amino acid synthesis.

**226.** (B) The active site cannot fully distinguish between the geometrically similar, uncharged $CO_2$ and $O_2$ molecules
The oxygenase activity of RuBisCO is a major limitation on photosynthetic efficiency, yet it is highly conserved across the plant kingdom. This activity is a result of the fundamental chemistry of the enzyme active site. Because carbon dioxide and molecular oxygen are both small, uncharged, and geometrically similar molecules, RuBisCO cannot perfectly distinguish between them. This evolutionary constraint means that the enzyme will always react with oxygen to some extent.

**227.** (C) By the binding of specific tightly-bound sugar phosphate inhibitors like CA1P (2-carboxyarabinitol 1-phosphate)
Many plants have evolved mechanisms to suppress RuBisCO activity during the night to avoid futile metabolic cycles. One common strategy involves the synthesis of specific sugar phosphate molecules that act as tight-binding inhibitors of the enzyme. A well-known example is 2-carboxyarabinitol 1-phosphate. These inhibitors bind to the active site of RuBisCO in the dark, effectively locking it in an inactive state. When light returns, these inhibitors are removed by RuBisCO activase.

**228.** (A) By using ATP hydrolysis to physically alter the conformation of RuBisCO, facilitating inhibitor release
RuBisCO activase is an essential enzyme that regulates the activity of RuBisCO in response to light and metabolic signals. Its primary function is to remove inhibitory sugar phosphates that frequently block the active site of RuBisCO. Using the energy from ATP hydrolysis, the activase physically remodels the structure of the RuBisCO enzyme. This conformational change reduces the affinity of RuBisCO for the inhibitor, causing its release and allowing the enzyme to become active.

**229.** (C) It increases relative to photosynthesis due to decreased $CO_2$ solubility and decreased specificity of RuBisCO for $CO_2$
As the temperature of a leaf increases, the rate of photorespiration rises more quickly than the rate of photosynthesis. This occurs for two main reasons. First, the solubility of carbon dioxide in water decreases more rapidly than the solubility of oxygen as temperatures climb. Second, the RuBisCO enzyme itself becomes less specific for carbon dioxide at higher temperatures. Together, these factors lead to a higher frequency of oxygenation events, reducing carbon gain.

**230.** (C) The C-terminal PRD (PAS-related domain) / Histidine kinase-related region
The movement of phytochromes from the cytoplasm to the nucleus is a critical step in light-induced signal transduction. This nuclear translocation is controlled by specific amino acid sequences known as nuclear localization signals. Within the phytochrome molecule, these critical signals are located in the C-terminal half of the protein. Specifically, they are found within the PAS-related domain and the histidine kinase-related region, which allow the active form to be recognized.

**231.** (A) Phytochrome A (phyA)
Phytochromes exhibit different physiological responses depending on the wavelength and intensity of light. Under conditions of continuous far-red light, a specific photoreceptor is primarily responsible for the plant development. This is phytochrome A. Unlike other phytochromes, phyA is uniquely capable of mediating the high irradiance response under far-red light. This allows seedlings to perceive light even under a dense canopy and initiate de-etiolation, which is essential for survival.

**232.** (B) It is rapidly degraded in the Pfr form, causing a continuous flux of newly synthesized, hypersensitive Pr molecules acting dynamically under FR
Phytochrome A is unique because of its extreme sensitivity and its rapid turnover in the light. In its active Pfr form, phyA is quickly targeted for degradation by the proteasome. Under continuous far-red light, a very small but constant population of active Pfr molecules is maintained. This continuous flux of newly synthesized Pr molecules being converted into Pfr is what drives the far-red high irradiance response, making phyA a highly dynamic sensor.

**233.** (A) A low Red to Far-Red (R:FR) ratio
Plants growing in crowded environments or under a canopy perceive the presence of neighbors through changes in the quality of light. Because

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(A) Solid carbon dioxide at $-78^{\circ}C$
(B) Deep freezers at $-80^{\circ}C$
(C) Refrigerators at $4^{\circ}C$
(D) Liquid nitrogen at $-196^{\circ}C$

2019

**48.** During cryopreservation, what is the primary cause of cell death that cryoprotectants aim to prevent?
(A) Intracellular ice crystal formation
(B) Rapid DNA degradation by endogenous nucleases
(C) Excessive buildup of ethylene gas
(D) Over-accumulation of starch grains

2001

**49.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Vitrification is a cryopreservation technique that avoids the formation of lethal ice crystals.
Reason (R): Vitrification involves the transition of aqueous cellular solutions directly into an amorphous, glassy state without freezing.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**50.** Consider the following statements about in vitro germplasm conservation:
I. Slow-growth storage relies on lowering the incubation temperature.
II. Osmotic agents like mannitol are added to reduce tissue growth rates.
III. It guarantees the complete elimination of somaclonal variation.
IV. It is ideal for short-to-medium term preservation of germplasm.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) I and II
(D) II, III and IV

2001

**51.** Which of the following vitamins is most critical in plant tissue culture media to support cellular metabolism and growth?
(A) Vitamin B1 (Thiamine)
(B) Vitamin C (Ascorbic acid)
(C) Vitamin K
(D) Vitamin D

2022

**52.** Among the various carbon sources, sucrose is universally preferred in plant tissue culture because:
(A) It is less expensive than all other sugars.
(B) It is highly thermostable during autoclaving.
(C) It efficiently translocates across cell membranes and easily breaks down into glucose and fructose.
(D) It acts as a potent growth regulator overriding hormonal signals.

2023

**53.** Match the macro-nutrients (List I) with their primary physiological functions (List II) in tissue culture media.
List I: (Macro-nutrient)
P. Nitrogen
Q. Phosphorus
R. Calcium
S. Magnesium
List II: (Physiological Function)
1. Cell wall synthesis and membrane functioning
2. Central component of the chlorophyll molecule
3. Synthesis of amino acids and nucleic acids
4. Energy transfer via ATP and structural part of nucleic acids
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-3, S-2

NODIA

2023

**54.** In the preparation of stock solutions for MS medium, calcium chloride ($CaCl_2$) is generally prepared separately from sulfates and phosphates to prevent:
(A) Volatilization of chlorine gas
(B) Rapid oxidation of micronutrients
(C) Spontaneous crystallization of sucrose
(D) Precipitation of insoluble calcium salts

2002

**55.** What is the fundamental principle behind using 70% ethanol rather than 100% (absolute) ethanol for surface sterilization?
(A) Absolute ethanol evaporates too slowly.
(B) Absolute ethanol fails to denature proteins effectively without the presence of water.
(C) Absolute ethanol initiates rapid somatic embryogenesis.

2025

**92.** Identify the correct pair of developmental stages and their characteristics in somatic embryogenesis.
(A) Globular stage - Appearance of distinct cotyledons
(B) Heart stage - Elongation of the embryonic axis
(C) Torpedo stage - Elongation of the embryonic axis
(D) Cotyledonary stage - Spherical mass of actively dividing cells

2010

**93.** Arrange the phases of plant cell suspension culture growth in their chronological sequence.
I. Log phase
II. Stationary phase
III. Lag phase
IV. Linear phase
(A) III, I, IV, II
(B) I, III, II, IV
(C) III, IV, I, II
(D) I, IV, III, II

2024

**94.** Consider the following statements regarding anther culture:
I. The pollen must be in the late uninucleate to early binucleate stage for optimal response.
II. Cold pretreatment of floral buds generally decreases androgenic response.
III. It results in the formation of haploid embryos or callus.
Which of the above statement(s) is/are correct?
(A) I and III
(B) I and II
(C) II and III
(D) I

2006

**95.** Colchicine exerts its polyploidizing effect by interacting directly with which cellular component?
(A) Actin microfilaments
(B) Tubulin dimers preventing microtubule polymerization
(C) DNA polymerase during the S phase
(D) Cellulose synthase in the plasma membrane

2016

**96.** What is the fundamental basis for producing virus-free plants through meristem tip culture?
(A) The meristem tip expresses specific antiviral chemicals.
(B) Viruses are unable to multiply in dark environments.
(C) Meristem cells completely lack plasmodesmata.
(D) The rate of cell division in the meristem exceeds the rate of viral replication and vascular connection is absent.

2015

**97.** Embryo rescue is most frequently applied to overcome which specific breeding challenge?
(A) Mitigating post-zygotic barriers in distant crosses
(B) Overcoming pre-zygotic self-incompatibility
(C) Inducing polyploidy in standard diploids
(D) Transferring chloroplast DNA directly

2008

**98.** Interspecific crosses between cultivated species and wild relatives often fail due to endosperm collapse. Embryo rescue bypasses this by:
(A) Artificially synthesizing endosperm around the seed.
(B) Directly fusing the gametes in a petri dish.
(C) Spraying the maternal parent with high doses of cytokinins.
(D) Excising the developing embryo and nourishing it on an artificial nutrient medium.

2023

**99.** Which tissue is excised and cultured specifically to produce triploid plants?
(A) Nucellus
(B) Endosperm
(C) Tapetum
(D) Suspensor

2008

**100.** Match the enzymes (List I) with their primary substrate targets (List II) during protoplast isolation.
List I: (Enzyme)
P. Cellulase
Q. Pectinase
R. Hemicellulase
S. Lipase
List II: (Substrate Target)
1. Middle lamella
2. Primary cell wall structural microfibrils
3. Cross-linking matrix polysaccharides
4. Lipid droplets/membranes
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-4, S-3
(C) P-3, Q-1, R-2, S-4
(D) P-2, Q-3, R-1, S-4

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(A) The developmental stage of the microspores at the time of excision.
(B) The presence of a strong artificial light source during initial incubation.
(C) The exclusion of all carbon sources from the induction medium.
(D) The age of the culture vessel used.

2011

**66.** In anther culture, albino plantlets frequently emerge instead of green plantlets. This phenomenon is predominantly reported in which family?
(A) Solanaceae
(B) Poaceae (Gramineae)
(C) Fabaceae
(D) Brassicaceae

2006

**67.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Ovary culture is an effective alternative for haploid production when anther culture fails.
Reason (R): Ovary culture directly induces the somatic layers of the ovary wall to undergo meiosis.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**68.** Intergeneric hybridization between wheat and rye leads to the production of Triticale. The primary reason for utilizing embryo rescue in this specific cross is:
(A) Premature shedding of the spike
(B) Degeneration of the endosperm leading to embryo starvation
(C) Complete lack of fertilization due to incompatible pollen
(D) Extreme toxicity of rye pollen on wheat stigmas

2024

**69.** Which enzyme formulation is most appropriate for degrading the hemicellulose fraction of the plant cell wall during protoplast isolation?
(A) Cellulase Onozuka R-10
(B) Rhozyme HP-150
(C) Pectolyase Y-23
(D) Macerozyme R-10

2019

**70.** Evans Blue is employed as a viability stain for plant cells and protoplasts. Based on its principle of action, which cells take up the dye?
(A) Living cells selectively transport it via ATP-dependent pumps.
(B) Dead cells absorb the dye because they have lost membrane integrity.
(C) Cells undergoing active mitosis accumulate it in the nucleus.
(D) Only fully differentiated cells absorb the dye.

2007

**71.** Match the cryoprotectants (List I) with their fundamental properties (List II).
List I: (Cryoprotectant)
P. DMSO
Q. Glycerol
R. Sucrose
S. Proline
List II: (Property)
1. Penetrating synthetic cryoprotectant
2. Non-penetrating sugar acting as an osmoticum
3. Naturally occurring amino acid providing stress tolerance
4. Penetrating polyol, highly viscous at low temperatures
(A) P-1, Q-4, R-2, S-3
(B) P-4, Q-1, R-3, S-2
(C) P-1, Q-2, R-4, S-3
(D) P-2, Q-3, R-1, S-4

NODIA

2019

**72.** During the chemical induction of protoplast fusion, high pH combined with high concentrations of a specific ion is often used. Which ion is this?
(A) $Ca^{\{2+\}}$
(B) $K^{+}$
(C) $Na^{+}$
(D) $Mg^{\{2+\}}$

2008

**73.** A heterokaryon, produced immediately following protoplast fusion, is defined by the presence of:
(A) Fused nuclei forming a single tetraploid nucleus.
(B) Completely integrated mitochondrial and nuclear genomes.
(C) One parental nucleus and one purely synthetic nucleus.
(D) Fused cytoplasm but distinct, unfused nuclei

(D) They replace the requirement for carbon sources in the medium.

2006

**110.** Which bioreactor type achieves liquid mixing and aeration by sparging air into a central draft tube, causing a cyclic flow of the suspension?
(A) Airlift bioreactor
(B) Stirred tank bioreactor
(C) Fluidized bed bioreactor
(D) Rotary drum bioreactor

2011

**111.** What is the primary advantage of utilizing calcium alginate beads for plant cell immobilization?
(A) It allows for the continuous recovery of secreted secondary metabolites without destroying the biomass.
(B) It permanently prevents cell division.
(C) It induces instantaneous somatic embryogenesis.
(D) It replaces the need for basic media nutrients.

2009

**112.** Consider the following statements concerning cryopreservation by vitrification:
I. It requires extremely rapid cooling rates.
II. It avoids the formation of intracellular ice completely.
III. Highly concentrated cryoprotective solutions (e.g., PVS2) are used.
IV. It relies on the formation of large extracellular ice crystals to dehydrate the cell.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I, II and IV
(C) II, III and IV
(D) I and III

2004

**113.** During slow-growth in vitro germplasm conservation, mannitol is added to the medium. What is its mechanistic role?
(A) It acts as a non-metabolizable osmotic agent, restricting water uptake and reducing growth rate.
(B) It functions as a rapid carbon source, keeping cells fully energized during cold storage.
(C) It chelates toxic heavy metals accumulating during extended storage.
(D) It solidifies the medium, replacing agar.

2022

**114.** Which specific stage of micropropagation (Stage I to IV) is most susceptible to significant losses due to desiccation and microbial attack?
(A) Stage I (Initiation)
(B) Stage II (Multiplication)
(C) Stage IV (Acclimatization)
(D) Stage III (Rooting)

2008

**115.** Nitsch's medium was formulated specifically for the successful in vitro culture of which specific plant tissues?
(A) Protoplasts of solanaceous species
(B) Anthers and microspores
(C) Meristem tips of orchids
(D) Endosperm of cereal crops

2018

**116.** Match the specific vitamins/supplements (List I) with their typical role or characteristic in tissue culture (List II).
List I: (Supplement)
P. Thiamine HCl
Q. Myo-inositol
R. Ascorbic acid
S. Glycine
List II: (Role/Characteristic)
1. Antioxidant to prevent tissue browning
2. Essential co-enzyme in carbohydrate metabolism
3. Amino acid occasionally added to support growth
4. Sugar alcohol crucial for membrane synthesis
(A) P-2, Q-4, R-1, S-3
(B) P-1, Q-4, R-2, S-3
(C) P-2, Q-1, R-4, S-3
(D) P-4, Q-2, R-1, S-3

2014

**117.** Which of the following carbon sources is generally considered the least effective for supporting standard plant tissue culture growth due to poor cellular uptake?
(A) Lactose
(B) Sucrose
(C) Glucose
(D) Fructose

2010

**118.** Which statement is an accurate reflection of the function of macronutrients in plant tissue culture media?
(A) Potassium is crucial for osmotic regulation but is not a structural component of any major biomolecule.
(B) Nitrogen is only required during the rooting phase.
(C) Phosphorus is primarily used for maintaining

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2007

**83.** Consider the following statements regarding slow-growth germplasm conservation:

I. It is typically conducted at standard culture temperatures ($25^{\circ}C$).

II. It increases the interval between necessary subcultures.

III. Abscisic acid (ABA) can be added to the medium to retard growth.

IV. It is considered a form of ex situ conservation.

Which of the above statement(s) is/are incorrect?

(A) I

(B) II and III

(C) I and IV

(D) III

2025

**84.** Which of the following is the most significant limitation of slow-growth storage compared to cryopreservation?

(A) Complete inability to recover live plants

(B) High risk of somaclonal variation accumulating over repeated subcultures

(C) Requirement for liquid nitrogen infrastructure

(D) Obligate need for continuous darkness

2015

**85.** The MS medium formulation is distinguished from many earlier media by its exceptionally high concentration of:

(A) Iron chelates

(B) Copper sulfate

(C) Nitrate and ammonium salts

(D) Sucrose

2025

**86.** Which vitamin is considered an absolute requirement (essential) in almost all plant tissue culture media formulations?

(A) Niacin (Nicotinic acid)

(B) Pyridoxine (Vitamin B6)

(C) Thiamine (Vitamin B1)

(D) Riboflavin (Vitamin B2)

2021

**87.** During surface sterilization, a surfactant is often added to the sterilizing solution. What is its exact purpose?

(A) To chemically degrade the plant cell wall

(B) To enhance contact between the sterilant and the plant surface by reducing surface tension

(C) To act as a buffer keeping the pH of the sterilant neutral

(D) To neutralize the toxicity of the sterilant post-exposure

2022

**88.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

Assertion (A): Filter sterilization is mandatory for preparing media containing gibberellic acid (GA3).

Reason (R): GA3 is thermolabile and its structural integrity is destroyed by the heat and pressure of an autoclave.

(A) Both A and R are true and R is the correct explanation of A.

(B) Both A and R are true but R is not the correct explanation of A.

(C) A is true but R is false.

(D) A is false but R is true.

2011

**89.** Which of the following statements most accurately describes 'Habituation' in plant tissue culture?

(A) The loss of the requirement for exogenous growth regulators by a previously dependent culture.

(B) The adaptation of plantlets to ex vitro greenhouse conditions.

(C) The process of rendering an explant totally pathogen-free.

(D) The accumulation of toxic phenolic exudates in the culture medium.

2005

**90.** In the context of organogenesis, if a callus culture is transferred to a medium containing 2.0 mg/L BAP and 0.1 mg/L NAA, what is the most likely morphological response?

(A) Shoot bud induction

(B) Extensive root proliferation

(C) Induction of somatic embryos

(D) Maintenance of undifferentiated callus growth

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2012

**91.** The primary purpose of Stage 0 in the micropropagation protocol is:

(A) Selection, maintenance, and pre-treatment of the mother plant

(B) Ex vitro rooting

(C) Production of synthetic seeds

(D) Rapid multiplication of adventitious shoots

2004

**127.** Which specific structure acts as the structural and nutritional support system during the early stages of in vitro zygotic embryo development, analogous to its role in vivo?
(A) Suspensor
(B) Tapetum
(C) Coleoptile
(D) Scutellum

2013

**128.** Consider the following primary pollen development pathways leading to androgenesis based on symmetrical vs asymmetrical division:
I. Pathway I: Symmetrical division of microspore
II. Pathway II: Asymmetrical division followed by division of vegetative cell
III. Pathway III: Asymmetrical division followed by division of generative cell
Which of the following statement(s) is/are correct?
(A) All three pathways can yield haploid embryos depending on the species.
(B) Only Pathway I yields haploids.
(C) Pathway III is the most common route in Poaceae.
(D) Pathway II exclusively produces triploids.

2010

**129.** Why is the stage of microspore development considered critical for the success of anther culture?
(A) Mature pollen grains (trinucleate) are generally recalcitrant and do not switch from a gametophytic to a sporophytic pathway.
(B) Tetrad stage microspores naturally double their chromosomes.
(C) Only mature pollen contains sufficient endogenous auxins.
(D) Binucleate pollen lacks a cell wall, making isolation easier.

2004

**130.** The physiological phenomenon of generating an embryo directly from an unfertilized egg cell within an ovule culture is known as:
(A) Parthenogenesis (a form of gynogenesis)
(B) Androgenesis
(C) Somatic embryogenesis
(D) Endospermogenesis

2022

**131.** Which crop species is a classic example of successfully applying embryo rescue to transfer disease resistance from a wild relative?
(A) Transfer of late blight resistance from Solanum demissum to cultivated potato.
(B) Transfer of CMS from radish to cabbage.
(C) Production of golden rice.
(D) Introduction of Bt toxin into cotton.

2006

**132.** In standard angiosperm development, the endosperm is triploid. This ploidy arises from the fusion of:
(A) One male gamete with the egg cell.
(B) One male gamete with two polar nuclei.
(C) Two male gametes with the polar nuclei.
(D) One male gamete with two synergid cells.

2019

**133.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Fluorescein diacetate (FDA) staining accurately distinguishes between living and dead protoplasts.
Reason (R): FDA is non-fluorescent until it is cleaved by intracellular esterases present only in living cells, releasing fluorescent fluorescein.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**134.** Which of the following sequences represents the correct general order for enzymatic protoplast isolation?
I. Incubation in enzyme mixture containing cellulase and pectinase
II. Surface sterilization of the source tissue
III. Peeling of the lower epidermis or slicing into fine strips
IV. Centrifugation and washing with osmoticum
(A) II, III, I, IV
(B) III, II, I, IV
(C) II, I, III, IV
(D) I, II, III, IV

2013

**135.** What is the precise function of applying a low-voltage alternating current (AC) field during protoplast electrofusion?
(A) It induces reversible pore formation in the plasma membrane.
(B) It forces the protoplasts into alignment, creating 'pearl chains' via dielectrophoresis.

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2005

**101.** The viability of isolated protoplasts can be confirmed using the Evans Blue exclusion test. In this test:
(A) Viable cells fluoresce bright green.
(B) Non-viable cells accumulate the blue dye.
(C) Viable cells undergo immediate division upon staining.
(D) The dye binds strictly to intact cell walls.

2003

**102.** Consider the following statements regarding chemical fusogens in protoplast fusion:
I. Polyethylene Glycol (PEG) acts by creating reversible bridges between adjacent membranes.
II. High concentrations of $Ca^{2+}$ at low pH strongly promote membrane fusion.
III. PEG-induced fusion is significantly enhanced when followed by elution with high pH, high $Ca^{2+}$ solution.
IV. Chemical fusion is strictly species-specific and fails in intergeneric crosses.
Which of the above statement(s) is/are incorrect?
(A) II and IV
(B) I and III
(C) II, III and IV
(D) I, II and IV

2014

**103.** In somatic hybridization, a "symmetric hybrid" refers to a regenerated plant that:
(A) Retains the full nuclear genome of both parental protoplasts.
(B) Contains only the nuclear genome of one parent.
(C) Possesses a strictly haploid genome from both parents.
(D) Contains only the chloroplasts of the donor species.

2022

**104.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Cybridization is a highly effective tool for transferring Cytoplasmic Male Sterility (CMS) across crop lines.
Reason (R): CMS traits are typically encoded by the mitochondrial genome, which is transferred intact during cybrid formation while nuclear genes are not mixed.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**105.** Point mutations resulting in somaclonal variation most frequently arise during which stage of tissue culture?
(A) Prolonged undifferentiated callus phase
(B) Ex vitro acclimatization
(C) Immediate isolation of the explant
(D) Cold storage of seeds

2006

**106.** In vitro selection for disease resistance typically involves supplementing the culture medium with:
(A) Purified host plant DNA
(B) Phytotoxins isolated from the target pathogen
(C) Live spores of the pathogenic fungi
(D) Broad-spectrum antibiotics

2010

**107.** The LD50 value determined prior to initiating in vitro mutagenesis refers to the mutagen dose that:
(A) Results in a 50% reduction in explant survival or cell viability.
(B) Induces mutations in 50% of the surviving population.
(C) Stimulates a 50% increase in secondary metabolite production.
(D) Doubles the chromosome number in 50% of the cells.

2003

**108.** The 'rol' genes situated on the Ri plasmid of Agrobacterium rhizogenes modulate the plant's sensitivity to which class of endogenous hormones?
(A) Auxins
(B) Gibberellins
(C) Ethylene
(D) Brassinosteroids

2023

**109.** Which of the following best describes the physiological function of abiotic elicitors like heavy metals in cell suspension cultures?
(A) They act as essential micronutrients boosting primary metabolism.
(B) They induce physiological stress, triggering defense pathways and secondary metabolite synthesis.
(C) They permanently arrest the cell cycle in the G2 phase.

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from both parents.

2019

**74.** Which strategy is most relevant for the selection of somatic hybrids after protoplast fusion?
(A) Culturing on a medium lacking carbon sources
(B) Using complementary biochemical mutants (auxotrophs)
(C) Lowering the incubation temperature to $4^{\circ}C$
(D) Subjecting the culture to complete darkness for 60 days

2008

**75.** Somaclonal variation is considered advantageous in plant breeding primarily when:
(A) Maintaining clonal fidelity of elite germplasm is the goal.
(B) Generating novel, unpredicted phenotypic variations is desired.
(C) Eradicating viruses from infected clones is required.
(D) Standardizing the protocol for synthetic seed production.

2017

**76.** Gametoclonal variation specifically refers to the variations observed in plants regenerated from:
(A) Leaf mesophyll protoplasts
(B) Root tip callus
(C) Gametic cells such as microspores
(D) Shoot apical meristems

2025

**77.** Which molecular mechanism is a primary cause of epigenetic somaclonal variation?
(A) Aneuploidy
(B) Activation of transposable elements
(C) DNA methylation shifts affecting gene expression
(D) Deletions of large chromosomal segments

2016

**78.** Identify the incorrect statement regarding hairy root cultures.
(A) They are characterized by a high degree of genetic stability.
(B) They require the constant exogenous addition of auxins for growth.
(C) They often produce secondary metabolites at levels equal to or higher than the mother plant.
(D) They exhibit plagiotropic (horizontal) and highly branched growth.

2022

**79.** In bioreactor design for plant cell suspensions, the "bubble column" bioreactor achieves mixing and aeration primarily through:
(A) Mechanical agitation using an impeller
(B) Sparging gas from the bottom of the vessel
(C) Rotation of the entire vessel around a horizontal axis
(D) Pumping the liquid through an external loop

2009

**80.** Consider the following statements regarding the addition of biotic elicitors to plant cell cultures:
I. They trigger the activation of the hypersensitive response and phytoalexin accumulation.
II. They inhibit all secondary metabolic pathways.
III. They cause an immediate transition from suspension to callus phase.
IV. They result in a drastic reduction in the synthesis of phenolic compounds.
Which of the above statement(s) is/are correct?
(A) I
(B) II
(C) III
(D) IV

2004

**81.** Precursor feeding is a strategy utilized in plant cell biotransformation. It involves:
(A) Providing a cheap nitrogen source to accelerate biomass accumulation.
(B) Adding an intermediate compound to the medium for specific structural modification by the cells.
(C) Supplementing the culture with high doses of physical mutagens.
(D) Using precursor cells (stem cells) to rejuvenate older cultures.

2017

**82.** What is the fundamental role of cryoprotectants like DMSO in the cryopreservation process?
(A) They lower the freezing point and minimize the formation of large, lethal intracellular ice crystals.
(B) They bind to DNA to prevent genetic mutations during storage.
(C) They serve as a permanent nutrient source during deep freeze storage.
(D) They induce a state of cellular respiration independent of oxygen.

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turgor pressure.
(D) Magnesium acts as the primary electron acceptor in respiration.

2003

**119.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Surface sterilants like sodium hypochlorite must be thoroughly rinsed off the explant with sterile distilled water before inoculation.
Reason (R): Residual sterilant on the explant surface can cause severe phytotoxicity and tissue death.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2015

**120.** In the context of organogenesis, root organogenesis is preferentially induced when the relative concentration of:
(A) Auxin is significantly higher than cytokinin.
(B) Cytokinin is significantly higher than auxin.
(C) Auxin and cytokinin are equal.
(D) Gibberellin dominates over auxin.

2001

**121.** What is the fundamental difference between cellular totipotency and pluripotency in plant systems?
(A) Totipotent cells have the capacity to regenerate into a complete, fertile plant, whereas pluripotent cells can only differentiate into specific tissue lineages.
(B) Pluripotent cells can regenerate a whole plant, while totipotent cells cannot.
(C) Totipotency is restricted to animal cells.
(D) Pluripotency only applies to mature xylem elements.

2024

**122.** During meristem tip culture, the explant size must be extremely small (0.1 - 0.5 mm) primarily to ensure:
(A) Complete elimination of viral pathogens.
(B) Rapid induction of roots.
(C) Direct somatic embryogenesis without callus.
(D) Survival during subsequent cryopreservation.

2015

**123.** Identify the incorrect statement regarding the application of thermotherapy.
(A) It involves exposing mother plants to elevated temperatures $(35 - 40^{\circ}C)$ for several weeks.
(B) It aims to inhibit viral replication and movement.
(C) It is frequently combined with meristem culture for higher efficiency.
(D) It relies on liquid nitrogen to shock-kill systemic viruses.

2003

**124.** The stationary phase in a cell suspension culture is characterized by:
(A) A rapid, exponential increase in total cell mass.
(B) A net zero growth rate where cell division equals cell death, often due to nutrient depletion.
(C) The maximum rate of primary metabolite production.
(D) Immediate lysis of all suspended cells.

2016

**125.** Which of the following describes the 'micro-chamber technique' for single-cell culture?
(A) Culturing cells in a massive 10,000 L industrial bioreactor.
(B) Isolating a single cell in a small droplet of medium on a specialized slide to observe continuous division.
(C) Plating cells on an agar surface to form isolated colonies.
(D) Suspending cells in calcium alginate beads for secondary metabolite harvest.

2008

**126.** Consider the following statements concerning synthetic seeds:
I. They can be transported and sown identically to true botanical seeds.
II. The encapsulating matrix provides a physical barrier and can be infused with nutrients and fungicides.
III. They are exclusively produced using zygotic embryos.
IV. Calcium alginate is the most preferred hydrogel for encapsulation.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) II, III and IV
(C) I, II and III
(D) I and IV

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(D) Absolute ethanol reacts violently with plant cuticles.

2011

**56.** Which of the following statements about autoclaving is least accurate?
(A) It operates on the principle of steam under pressure.
(B) It destroys vegetative cells but is ineffective against bacterial endospores.
(C) The standard sterilization cycle normally lasts for 15-20 minutes.
(D) Prolonged autoclaving can lead to the caramelization of sucrose.

2024

**57.** Redifferentiation in tissue culture implies the physiological process where:
(A) Meristematic callus cells mature and specialize into distinct plant organs.
(B) Differentiated cells revert back to undifferentiated mass.
(C) Organelles are exchanged between two different species.
(D) The chromosome number of the explant is halved.

2018

**58.** Identify the incorrect pair regarding the developmental stages of micropropagation.
(A) Stage 0 - Selection and preparation of the mother plant
(B) Stage I - Establishment of aseptic culture
(C) Stage II - Hardening and acclimatization of plantlets
(D) Stage III - Rooting of multiplied shoots

2007

**59.** Thermotherapy is widely utilized in micropropagation for the elimination of:
(A) Agrobacterium spp.
(B) Viral pathogens
(C) Fungal spores
(D) Nematodes

2021

**60.** During the linear phase of cell suspension culture growth kinetics, what cellular activity is most prominent?
(A) Adaptation to the new nutrient environment
(B) Exponential doubling of the cell population
(C) Constant rate of cell division coupled with steady cell expansion
(D) Accumulation of toxic metabolites and rapid cell death

2001

**61.** Identify the correct statement regarding single-cell culture using Bergmann's plating technique.
(A) Cells are mixed with warm, liquid agar medium and spread in a thin layer in a Petri dish.
(B) Single cells are suspended directly in liquid medium and agitated vigorously.
(C) Single cells are injected directly into a host plant stem.
(D) It requires the compulsory use of a nurse culture to support division.

2016

**62.** Which embryogenic stage in dicots is characterized by the initiation of cotyledon primordia?
(A) Globular stage
(B) Heart stage
(C) Torpedo stage
(D) Suspensor stage

2016

**63.** Consider the following statements concerning somatic embryogenesis:
I. It originates from vegetative cells rather than gametic fusion.
II. Somatic embryos possess a distinct vascular connection with the maternal tissue.
III. They exhibit bipolarity, possessing both a shoot and a root pole.
IV. It can be induced by high concentrations of 2,4-D in the medium.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I and III

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2009

**64.** Consider the following steps used in the encapsulation-dehydration protocol for cryopreservation:
I. Air desiccation to lower water content
II. Encapsulation in calcium alginate beads
III. Immersion in liquid nitrogen
IV. Pre-culture in high-sucrose medium
What is the correct sequential order of these steps?
(A) II, IV, I, III
(B) IV, II, I, III
(C) II, I, IV, III
(D) I, II, IV, III

2005

**65.** The primary factor influencing the success of anther culture in yielding haploids is:

(C) It denatures the cell wall synthesizing enzymes.
(D) It permanently fuses the nuclear membranes.

2012

**136.** Which selection mechanism for somatic hybrids relies on the inability of the parental lines to grow on a specific basal medium without supplementation, whereas the hybrid can?
(A) Visual selection based on pigmentation
(B) Antibiotic resistance complementation
(C) Auxotrophic mutant complementation
(D) Isozyme marker analysis

2006

**137.** Which of the following best defines a "cybrid"?
(A) A cytoplasmic hybrid containing a hybrid cytoplasm but only one parental nucleus.
(B) A hybrid cell containing the full nuclear and cytoplasmic genomes of both parents.
(C) A somatic embryo derived from endosperm tissue.
(D) A plant cell engineered via Agrobacterium transformation.

2008

**138.** Which statement regarding epigenetic somaclonal variation is most accurate?
(A) It involves permanent changes in the DNA base sequence.
(B) It is strictly caused by gross chromosomal aberrations.
(C) It is often reversible and involves modifications such as DNA methylation or histone acetylation.
(D) It only occurs when chemical mutagens are added to the medium.

2018

**139.** Consider the following statements concerning in vitro mutagenesis:
I. EMS (Ethyl methanesulfonate) primarily induces point mutations, specifically GC to AT transitions.
II. Gamma irradiation generally causes extensive DNA breakage and chromosomal deletions.
III. The $M1$ generation in tissue culture immediately reveals recessive mutations.
IV. Chimerism is a common issue when multicellular explants are mutagenized.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I and II

2005

**140.** In screening for mutant cell lines resistant to a specific herbicide, the optimal selective strategy involves:
(A) Incorporating the herbicide into the medium at a concentration slightly above the established lethal dose for wild-type cells.
(B) Culturing cells on a medium entirely devoid of the herbicide.
(C) Adding the herbicide strictly during the final acclimatization phase.
(D) Treating the mother plant with the herbicide prior to explant excision.

2012

**141.** The use of biotic elicitors to enhance secondary metabolite production often mimics which natural plant interaction?
(A) Plant-pathogen interactions leading to phytoalexin synthesis
(B) Mutualistic symbiosis with nitrogen-fixing bacteria
(C) Phototropism in response to directional light
(D) Gravitropism in root tips

2016

**142.** Agrobacterium rhizogenes infects wounded plant tissues and transfers a segment of its Ri plasmid into the plant genome. What does "Ri" stand for?
(A) Recombinant integration
(B) Resistance inducing
(C) Root inducing
(D) Regeneration independent

2021

**143.** Match the bioreactor types (List I) with their primary operational mechanism (List II) for plant cell cultures.
List I: (Bioreactor Type)
P. Stirred tank
Q. Bubble column
R. Airlift
S. Rotating drum
List II: (Operational Mechanism)
1. Mixing achieved strictly by sparging gas from the base without internal loops
2. Mechanical mixing via a rotating impeller
3. Mixing via a gas-driven cyclic liquid flow through a draft tube
4. Mixing achieved by the physical rotation of a horizontal cylindrical vessel
(A) P-2, Q-1, R-3, S-4

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for scaling up plant cell suspensions.
Reason (R): Plant cells are highly sensitive to hydrodynamic shear forces generated by mechanical impellers used in stirred tanks.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**179.** Immobilized plant cells used in biotransformation often outlive freely suspended cells. This increased longevity is primarily attributed to:
(A) The physical protection against shear stress provided by the matrix.
(B) The total cessation of cellular respiration.
(C) The exclusion of all water from the immobilizing matrix.
(D) The spontaneous doubling of their chromosome number.

2016

**180.** During vitrification for cryopreservation, what is the specific purpose of the highly concentrated Plant Vitrification Solution?
(A) To severely dehydrate the cells and increase cytoplasmic viscosity, ensuring a glass transition upon freezing.
(B) To induce rapid cell division before freezing.
(C) To chemically mutate the genome for cold tolerance.
(D) To break down the cell wall for easier freezing.

2004

**181.** Which chemical is universally utilized as the standard permeating cryoprotectant in almost all slow-freezing plant cryopreservation protocols?
(A) Dimethyl sulfoxide (DMSO)
(B) Polyethylene glycol (PEG)
(C) Sodium alginate
(D) Ethidium bromide

2013

**182.** Consider the following statements regarding in vitro minimal-growth storage:
I. It is heavily utilized for the conservation of vegetatively propagated crops like potato and banana.
II. Storage temperatures are typically reduced to $4 - 15^{\circ} C$ depending on the species.
III. It guarantees indefinite preservation without any need for subculturing.
IV. Osmotic retardants like sucrose or mannitol may be increased in the medium.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I and III

2002

**183.** Which component of the MS medium serves as the primary source of essential reduced nitrogen?
(A) Ammonium nitrate
(B) Potassium nitrate
(C) Calcium chloride
(D) Potassium dihydrogen phosphate

2022

**184.** Identify the incorrect statement regarding the role of vitamins in tissue culture media.
(A) Thiamine is considered essential for most plant cell cultures.
(B) Nicotinic acid and pyridoxine often enhance growth but are not universally essential.
(C) Vitamins function primarily as co-factors for critical enzymatic reactions.
(D) Myo-inositol is a sugar alcohol crucial for membrane synthesis.

2015

**185.** During the preparation of MS media stock solutions, micronutrients are generally concentrated 100x or 1000x. Which of the following is considered a micronutrient?
(A) $ZnSO_4$
(B) $KH_2PO_4$
(C) $MgSO_4$
(D) $CaCl_2$

2019

**186.** Sequence the standard steps for preparing filter-sterilized heat-labile plant growth regulators (PGRs) for inclusion into agar-solidified media.
I. Autoclave the basal agar medium and allow it to cool to approximately $45 - 50^{\circ} C$
II. Prepare the PGR solution and pass it through a sterile 0.22 $\mu m$ membrane filter
III. Aseptically add the filtered PGR solution to the warm, liquid agar medium
IV. Mix gently and pour into sterile culture vessels
(A) II, I, III, IV
(B) I, II, III, IV
(C) II, III, I, IV
(D) I, III, II, IV

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(B) PEG (Polyethylene glycol)
(C) DMSO (Dimethyl sulfoxide)
(D) Tris-HCl

2009

**153.** A researcher uses a 0.22 $\mu m$ membrane filter for sterilizing a heat-sensitive vitamin solution. This pore size is specifically chosen because it effectively blocks:
(A) Most bacteria and fungal spores
(B) All known viruses
(C) Only large fungal mycelia
(D) Dissolved chemical salts

2003

**154.** What is the standard concentration range and exposure time for using sodium hypochlorite (commercial bleach) for surface sterilization of resilient explants like seeds or woody stems?
(A) 0.01% available chlorine for 5 minutes
(B) 0.1% available chlorine for 1 minute
(C) 1-5% available chlorine for 10-20 minutes
(D) 10-15% available chlorine for 60 minutes

2023

**155.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Cellular totipotency implies that isolated plant cells can be directed to form any plant organ given the right hormonal cues.
Reason (R): The Skoog-Miller model demonstrated that exogenous ratios of auxins and cytokinins control the developmental fate of undifferentiated callus cells.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**156.** In the context of micropropagation, "hyperhydricity" is an undesirable physiological disorder characterized by:
(A) Tissues appearing water-soaked, translucent, and possessing poor stomatal function.
(B) Over-lignification of the stem making the plantlets excessively rigid.
(C) The production of heavily pigmented red leaves.
(D) Immediate shedding of all root structures.

2003

**157.** During the acclimatization phase (Stage IV), plantlets are gradually exposed to ex vitro conditions. The primary physiological adaptations required during this phase are:
(A) Developing a functional waxy cuticle and capable stomatal regulatory mechanisms.
(B) Transitioning to heterotrophic growth and losing chlorophyll.
(C) Shedding the primary root system to form a taproot.
(D) Halving their chromosome number to adapt to natural light.

2014

**158.** Which of the following defines "lag phase" in a freshly subcultured plant cell suspension?
(A) The period of maximum secondary metabolite accumulation.
(B) The initial period of adaptation where cell mass increases without significant cell division.
(C) A period of rapid exponential cell division.
(D) The phase where cell death exceeds cell division.

2014

**159.** In Bergmann's cell plating technique, the base medium is solidified using a low concentration of agar (usually around 0.6%). Why is this specific concentration chosen?
(A) To allow single cells to remain suspended yet spatially separated while still permitting nutrient diffusion.
(B) To completely prevent oxygen diffusion.
(C) To act as a strong physical mutagen.
(D) To selectively kill bacterial contaminants.

2019

**160.** Sequence the generalized events occurring during indirect somatic embryogenesis.
I. Transfer to auxin-free or low-auxin medium for embryo development
II. Selection of explant
III. Induction of embryogenic callus on high-auxin (e.g., 2,4-D) medium
IV. Maturation and germination of somatic embryos
(A) II, III, I, IV
(B) II, I, III, IV
(C) III, II, I, IV
(D) II, III, IV, I

2018

**161.** The encapsulation matrix for artificial seeds primarily consists of sodium alginate. This

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androgenesis induction across most cereal species?
(A) Late uninucleate to early binucleate stage
(B) Early meiosis I
(C) Tetrad stage
(D) Mature trinucleate pollen grain

2006

**198.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Cold pretreatment ($4^{\circ}C$ for several days) of floral buds prior to anther excision significantly enhances the frequency of androgenesis.
Reason (R): Cold stress alters the normal gametophytic developmental pathway, delaying pollen maturation and promoting symmetrical division of the microspore.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**199.** Consider the following statements concerning embryo rescue:
I. It is frequently used to recover hybrids from interspecific incompatible crosses.
II. Immature embryos require a more complex nutrient medium compared to mature embryos.
III. It guarantees that the resulting hybrid will be fully fertile.
IV. The excision process must be performed under strictly aseptic conditions.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I and IV

2024

**200.** The principal advantage of producing triploid plants via endosperm culture in commercial fruit crops is:
(A) Production of completely seedless fruits due to highly irregular meiosis.
(B) Enhanced resistance to all soil-borne nematodes.
(C) Doubling of the photosynthetic rate per leaf area.
(D) Ability to fix atmospheric nitrogen.

2016

**201.** Match the protoplast fusion methods (List I) with their fundamental mechanisms (List II).
List I: (Fusion Method)
P. High pH / High $Ca^{2+}$
Q. PEG treatment
R. Electrofusion
S. Dextran sulfate
List II: (Mechanism)
1. Membrane agglutination followed by fusion upon dilution
2. Dielectrophoresis and reversible membrane breakdown
3. Neutralization of surface charge and bridge formation at basic pH
4. Polyanion-induced electrostatic interaction
(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-1, R-4, S-3

2016

**202.** What is the fundamental purpose of incorporating an osmoticum into the enzyme solution during protoplast isolation?
(A) To cause slight plasmolysis, shrinking the protoplast away from the cell wall and preventing osmotic lysis.
(B) To trigger the immediate cell wall regeneration process.
(C) To act as a direct carbon source for rapid respiration.
(D) To denature endogenous nucleases that might destroy the protoplast DNA.

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2006

**203.** Identify the incorrect statement regarding symmetric somatic hybridization.
(A) It is extensively used to transfer partial organelle genomes without touching the nucleus.
(B) It involves the complete fusion of two intact protoplasts.
(C) The resulting hybrid possesses the sum of the chromosome numbers of both parents.
(D) Segregation and elimination of chromosomes can occasionally occur spontaneously.

2015

**204.** Which selection strategy for somatic hybrids involves one parent being resistant to antibiotic A, and the other being resistant to antibiotic B, followed by culturing fused cells on media containing both antibiotics?
(A) Double mutant complementation

fusion
(D) Fluorescein Diacetate - Viability testing

2013

**170.** Consider the following statements concerning protoplast electrofusion:
I. Dielectrophoresis aligns the protoplasts into pearl chains.
II. A high voltage DC pulse causes reversible membrane breakdown Facilitating fusion.
III. It is generally more toxic to cells than chemical fusion using PEG.
IV. It allows for the microscopic observation and targeted fusion of specific cell pairs.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I and IV

2016

**171.** A major advantage of somatic hybridization over conventional sexual hybridization is that it:
(A) Allows for the combination of genomes from sexually incompatible species.
(B) Strictly prevents the transfer of cytoplasmic organelles.
(C) Requires matching ploidy levels between the parental species.
(D) Eliminates the need for any form of tissue culture regeneration.

2024

**172.** The transfer of herbicide resistance linked to the chloroplast genome from a wild species to a cultivated crop is best achieved through:
(A) Cybridization
(B) Anther culture
(C) Meristem tip culture
(D) Hairy root culture

2005

**173.** Somaclonal variation is primarily assessed at the molecular level to distinguish it from transient physiological adaptations. Which technique is commonly utilized for this?
(A) Molecular marker analysis (e.g., RAPD, AFLP, SSR)
(B) Measuring total fresh weight
(C) Assessing leaf stomatal index
(D) Visual inspection of flower color

2019

**174.** Which of the following is an example of an in vitro selection strategy to develop biotic stress resistant lines?
(A) Culturing cells in media supplemented with culture filtrates from a pathogenic fungus.
(B) Culturing callus on media containing high concentrations of sodium chloride.
(C) Irradiating explants with gamma rays.
(D) Encapsulating embryos in calcium alginate.

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2022

**175.** When determining the lethal dose (LD50) of a chemical mutagen like EMS in a plant tissue culture system, what does the resulting value specifically indicate?
(A) The dose that inhibits 50% of the growth or causes 50% mortality in the treated explant population.
(B) The exact dose that will yield 50% useful mutants.
(C) The percentage of the genome that has been mutated.
(D) The time required to reach the stationary phase.

2004

**176.** Identify the incorrect statement regarding the production of secondary metabolites in vitro.
(A) Undifferentiated callus cultures frequently produce lower yields of secondary metabolites compared to intact source plants.
(B) Elicitation is a common strategy to enhance target metabolite yields.
(C) Precursor feeding can push metabolic flux toward the desired final product.
(D) Immobilization of cells generally decreases the rate of secondary metabolite secretion to zero.

2005

**177.** In the context of secondary metabolite production, what is the role of a 'two-stage' culture system?
(A) Stage 1 optimizes biomass production; Stage 2 optimizes product synthesis.
(B) Stage 1 involves protoplast fusion; Stage 2 involves cybrid selection.
(C) Stage 1 occurs entirely in the dark; Stage 2 occurs entirely under UV light.
(D) Stage 1 uses solid media; Stage 2 uses strictly soil-based systems.

2024

**178.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Airlift bioreactors are generally preferred over standard stirred-tank bioreactors

(B) P-1, Q-2, R-3, S-4
(C) P-2, Q-3, R-1, S-4
(D) P-3, Q-1, R-4, S-2

2018

**144.** Polyurethane foam is sometimes used for plant cell immobilization. What is its structural classification as an immobilization matrix?
(A) Porous support matrix for adsorption/ attachment
(B) Gel entrapment matrix
(C) Liquid-liquid partition membrane
(D) Covalent cross-linking agent

2019

**145.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Biotransformation using plant cell cultures can produce novel stereo-specific compounds difficult to synthesize chemically.
Reason (R): Plant cells possess specific enzymes that can catalyze complex reactions like hydroxylation, glycosylation, and methylation on exogenous substrates.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**146.** Which cryopreservation technique relies on embedding explants in alginate beads followed by severe desiccation prior to freezing?
(A) Vitrification
(B) Encapsulation-dehydration
(C) Slow-freezing
(D) Droplet-vitrification

2007

**147.** What is the fundamental difference between slow-freezing and rapid-freezing protocols in cryopreservation?
(A) Slow-freezing allows for controlled extracellular ice formation causing protective cellular dehydration, while rapid-freezing prevents ice crystal formation entirely through vitrification.
(B) Slow-freezing is used exclusively for protoplasts; rapid-freezing for whole organs.
(C) Slow-freezing does not require cryoprotectants.
(D) Rapid-freezing is conducted at $-20^{\circ}C$.

2016

**148.** Minimal-growth storage techniques for in vitro germplasm conservation are primarily designed to:
(A) Extend the subculture interval by reducing the cellular metabolic rate.
(B) Induce rapid somatic embryogenesis.
(C) Eradicate systemic viral infections.
(D) Produce large quantities of secondary metabolites.

2023

**149.** Identify the incorrect statement regarding plant tissue culture repositories.
(A) They ensure the safe duplication and ex situ conservation of elite germplasm.
(B) They mandate frequent subculturing (every week) to maintain strict genetic stability.
(C) They often utilize cold storage $(4-10^{\circ}C)$ for slow-growth maintenance.
(D) They serve as a critical backup for field gene banks susceptible to natural disasters.

2021

**150.** Which carbon source requires autoclaving caution due to potential caramelization and breakdown into toxic byproducts if heated excessively?
(A) Fructose
(B) Sucrose
(C) Starch
(D) Myo-inositol

2022

**151.** Consider the following statements concerning the functions of essential micro-nutrients in tissue culture:
I. Iron is a critical component of cytochromes and is essential for chlorophyll synthesis.
II. Boron is involved in cell wall synthesis and membrane integrity.
III. Molybdenum is a cofactor for nitrate reductase.
IV. Zinc completely substitutes for calcium in membrane stabilization.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I, III and IV
(C) II, III and IV
(D) I and II

2025

**152.** Which chelating agent is conventionally paired with iron ($FeSO_4$) in MS medium to ensure iron remains soluble and bioavailable over a wide pH range?
(A) EDTA (Ethylenediaminetetraacetic acid)

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2025

**187.** Mercuric chloride ($HgCl_2$) is an extremely potent sterilant. Its microbicidal activity is primarily due to its ability to:
(A) Coagulate and precipitate cellular proteins by interacting with sulfhydryl groups.
(B) Dissolve the lipid bilayer completely.
(C) Act as a competitive inhibitor of DNA polymerase.
(D) Rapidly oxidize the cell wall cellulose.

2007

**188.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Dedifferentiation is a prerequisite for the induction of a callus from mature, specialized tissues like leaves.
Reason (R): Dedifferentiation involves the mature cells losing their specialized structures and functions to re-enter the cell cycle and regain meristematic activity.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**189.** According to the Skoog-Miller concept, if an explant is cultured on a medium containing equal and moderate levels of both auxin and cytokinin, the most probable developmental outcome is:
(A) Unorganized callus proliferation
(B) Exclusively root formation
(C) Exclusively shoot formation
(D) Immediate cell death

2018

**190.** In the context of micropropagation, the production of protocorm-like bodies (PLBs) is most analogous to:
(A) Somatic embryos
(B) Zygotic embryos
(C) Adventitious roots
(D) Endosperm nodules

2018

**191.** Stage III of micropropagation is specifically dedicated to:
(A) Rooting of the in vitro generated shoots
(B) Establishment of the aseptic explant
(C) Multiplication of propagules
(D) Field transfer and hardening

2008

**192.** Which thermotherapy protocol is most commonly applied to eradicate viruses from entire mother plants prior to meristem excision?
(A) Alternating cycles of $-20^{\circ}C$ and $4^{\circ}C$
(B) Cultivation in a growth chamber at $35-40^{\circ}C$ for 3 to 4 weeks
(C) Plunging roots into boiling water ($100^{\circ}C$) for 30 seconds
(D) Exposure to $121^{\circ}C$ for 15 minutes

2006

**193.** Identify the incorrect statement regarding cell suspension growth kinetics.
(A) Lag phase - Preparation for cell division, ATP accumulation
(B) Log phase - Maximum cell division rate
(C) Stationary phase - Exponential increase in primary metabolites
(D) Decline phase - Senescence and cell lysis

2007

**194.** Somatic embryogenesis differs fundamentally from organogenesis in that somatic embryos:
(A) Exhibit bipolarity, possessing both an apical and a basal meristem simultaneously.
(B) Originate strictly from the epidermal layer.
(C) Are always physically attached to the maternal vascular system.
(D) Require strictly auxin-free media for initiation.

2017

**195.** Which auxin is most widely utilized and generally considered indispensable for the induction of somatic embryogenesis in a wide range of species?
(A) 2,4-D (2,4-Dichlorophenoxyacetic acid)
(B) IAA (Indole-3-acetic acid)
(C) IBA (Indole-3-butyric acid)
(D) NAA (1-Naphthaleneacetic acid)

2004

**196.** The desiccation of artificial seeds prior to storage serves to:
(A) Induce a state of quiescence similar to natural orthodox seeds.
(B) Kill any contaminating bacteria.
(C) Irreversibly denature the encapsulation matrix.
(D) Trigger the premature germination of the radicle.

2015

**197.** Which specific stage of microspore development is generally considered most responsive to

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matrix is polymerized by dropping it into a solution of:
(A) Calcium chloride
(B) Potassium nitrate
(C) Sodium hydroxide
(D) Magnesium sulfate

2008

**162.** Identify the incorrect statement regarding artificial (synthetic) seeds.
(A) They provide a means for direct field sowing of clonally propagated elite plants.
(B) The encapsulation matrix cannot support the addition of any beneficial microbes or mycorrhizae.
(C) They are highly useful for propagating male-sterile lines.
(D) Desiccation-tolerant synthetic seeds can be stored for extended periods.

2004

**163.** In producing doubled haploids via anther culture, colchicine treatment is often preferred over spontaneous doubling because:
(A) Spontaneous doubling occurs at very low and unpredictable frequencies.
(B) Colchicine simultaneously induces resistance to fungal pathogens.
(C) Colchicine guarantees the formation of hexaploid plants.
(D) Spontaneous doubling results entirely in chimeral plants.

2017

**164.** What physiological limitation is most frequently encountered in gynogenesis compared to androgenesis?
(A) The frequency of responding ovules is generally much lower than the vast number of responsive microspores per anther.
(B) Gynogenesis strictly produces triploid plants.
(C) Ovaries cannot be surface sterilized effectively.
(D) Unfertilized ovules naturally lack a haploid egg cell.

2008

**165.** Match the specialized culture techniques (List I) with their defining primary application (List II).
List I: (Culture Technique)
P. Meristem culture
Q. Endosperm culture
R. Embryo rescue
S. Anther culture
List II: (Primary Application)
1. Overcoming post-zygotic incompatibility
2. Producing virus-free planting material
3. Producing homozygous doubled haploids
4. Generating seedless triploid plants
(A) P-2, Q-4, R-1, S-3
(B) P-1, Q-3, R-2, S-4
(C) P-2, Q-1, R-4, S-3
(D) P-4, Q-2, R-1, S-3

2018

**166.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Interspecific hybridization often fails due to the precocious abortion of the developing embryo.
Reason (R): The endosperm fails to develop properly due to genomic imbalances between the parent species.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**167.** Endosperm tissue is generally recalcitrant to standard organogenesis. However, when successfully cultured, it most commonly regenerates via:
(A) Callus induction followed by shoot organogenesis
(B) Direct somatic embryogenesis
(C) Spontaneous doubling leading to hexaploidy
(D) Generation of haploid microspores

2002

**168.** Which parameter is considered absolutely critical for maintaining the stability of naked protoplasts during the enzymatic isolation process?
(A) The maintenance of an iso-osmotic or slightly hypertonic environment using osmotica.
(B) The inclusion of a strong auxin like 2,4-D.
(C) The complete exclusion of all nitrogen sources.
(D) Agitation at speeds exceeding 250 rpm.

2015

**169.** Identify the incorrect statement regarding protoplast technology.
(A) Cellulase - Digests primary cell wall
(B) Pectinase - Digests middle lamella
(C) Mannitol - Induces spontaneous membrane

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(B) Visual marker selection
(C) Auxotrophic complementation
(D) Epigenetic screening

2024

**205.** Somaclonal variation is thought to arise from various genetic and epigenetic mechanisms. Which of the following is specifically a genetic mechanism?
(A) Chromosomal translocations and aneuploidy
(B) DNA methylation changes
(C) Histone deacetylation
(D) Transient silencing of a transgene

2004

**206.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): In vitro selection using fusaric acid can successfully yield disease-resistant plant lines.
Reason (R): Fusaric acid is a non-specific herbicide that randomly mutates plant DNA to create resistance genes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**207.** In the context of in vitro mutagenesis, which of the following represents a physical mutagen commonly used to irradiate calli or seeds?
(A) Gamma rays from a Cobalt-60 source
(B) EMS (Ethyl methanesulfonate)
(C) MMS (Methyl methanesulfonate)
(D) Sodium azide

2009

**208.** Hairy root cultures are distinguished from standard unorganized root cultures primarily by their:
(A) Plagiotropic growth habit and lack of geotropism.
(B) Strict dependence on exogenous cytokinins.
(C) Complete lack of secondary metabolite production.
(D) Inability to survive in liquid suspension media.

2001

**209.** Consider the following statements regarding the use of elicitors in plant cell culture:
I. Elicitors signal the plant cell to activate defense-related secondary metabolic pathways.
II. Fungal cell wall homogenates serve as potent biotic elicitors.
III. Elicitation is usually performed during the lag phase to maximize biomass.
IV. UV light and heavy metal salts act as abiotic elicitors.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I and IV

2011

**210.** Which bioreactor design is characterized by a central vertical tube that physically separates the upward flow of sparged liquid from the downward flow of de-gassed liquid?
(A) Airlift bioreactor
(B) Stirred-tank bioreactor
(C) Rotary drum bioreactor
(D) Membrane bioreactor

2009

**211.** What is the main objective of employing plant cell immobilization techniques in industrial biotechnology?
(A) To facilitate continuous operation and easy separation of cells from the product-containing medium.
(B) To permanently halt all metabolic activities.
(C) To induce rapid cellular dehydration.
(D) To increase the frequency of somatic crossing over.

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2016

**212.** Cryoprotectants are vital for successful cryopreservation. How does a penetrating cryoprotectant like DMSO differ functionally from a non-penetrating osmoticum like sucrose?
(A) DMSO enters the cell to reduce the freezing point and prevent internal ice, whereas sucrose acts externally to osmotically dehydrate the cell.
(B) DMSO entirely replaces the cell's water, whereas sucrose prevents water from escaping.
(C) DMSO is highly toxic and kills the cell, whereas sucrose acts as a nutrient.
(D) DMSO forms a solid physical shell around the cell wall, whereas sucrose remains liquid.

2013

**213.** Sequence the critical steps involved in a standard vitrification protocol for shoot tips.
I. Excision of the shoot tip
II. Plunging rapidly into liquid nitrogen
III. Pre-culture on medium enriched with sucrose
IV. Exposure to highly concentrated Plant Vitrification Solution (PVS2)
(A) I, III, IV, II
(B) I, IV, III, II
(C) III, I, IV, II
(D) I, II, III, IV

2002

**214.** Identify the incorrect statement concerning slow-growth germplasm storage.
(A) It requires extensive infrastructure similar to liquid nitrogen cryogenics.
(B) Growth is deliberately retarded using lower temperatures or osmotic agents.
(C) It significantly reduces the frequency of required subcultures compared to standard maintenance.
(D) It is particularly suited for maintaining clonal collections of tuber crops.

2013

**215.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): The management of a plant tissue culture repository requires strict indexing for latent viral infections prior to long-term storage.
Reason (R): Cold storage and cryopreservation do not eradicate viruses.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001

**216.** In the formulation of MS medium, which compound is specifically added to provide the essential micronutrient Boron?
(A) $H_3BO_3$ (Boric acid)
(B) $Na_2B_4O_7$ (Borax)
(C) $CuSO_4$
(D) $CoCl_2$

2010

**217.** When optimizing a culture medium, if tissue browning and necrosis are observed due to the oxidation of phenolic compounds, which specific additive is most commonly utilized to mitigate this?
(A) Activated charcoal or ascorbic acid
(B) Extra sucrose
(C) High concentrations of 2,4-D
(D) Polyethylene glycol

2012

**218.** Filter sterilization is strictly required for preparing solutions of:
(A) Antibiotics like kanamycin and hygromycin
(B) Agar and Gelrite
(C) Macronutrient salts
(D) Sucrose

2014

**219.** Identify the incorrect statement regarding the application of chemical surface sterilants.
(A) Mercuric chloride requires zero rinsing because it is highly volatile and non-toxic.
(B) The optimal exposure time varies significantly depending on the concentration of the sterilant and the toughness of the explant.
(C) Ethanol (70%) is often used as a brief initial rinse prior to a stronger sterilant.
(D) Sodium hypochlorite solutions lose their efficacy rapidly if left open to light and air.

2011

**220.** Match the micropropagation stages (List I) with their defining actions (List II).
List I: (Micropropagation Stage)
P. Stage I
Q. Stage II
R. Stage III
S. Stage IV
List II: (Defining Action)
1. Profuse multiplication of axillary or adventitious shoots
2. Transfer from heterotrophic in vitro conditions to autotrophic greenhouse conditions
3. Surface sterilization and initiation of aseptic culture
4. Transfer of shoots to auxin-rich media for root induction
(A) P-3, Q-1, R-4, S-2
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-2, Q-1, R-4, S-3

2003

**221.** Which specific pathogen elimination technique utilizes the principle that the vascular system

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of the apical dome is entirely absent or not fully developed?
(A) Meristem tip culture
(B) Chemotherapy using ribavirin
(C) Thermotherapy
(D) Protoplast fusion

2010

**222.** In establishing a cell suspension culture from a friable callus, what physical mechanism is primarily used to disperse the cells into a liquid medium?
(A) High-frequency sonication
(B) Repeated freezing and thawing
(C) Continuous agitation on an orbital shaker
(D) Application of a high voltage electrical field

2016

**223.** Consider the following statements regarding single-cell culture:
I. Single cells can be isolated mechanically or enzymatically.
II. The micro-chamber technique utilizes a nurse tissue to supply unknown complex growth factors to the single cell.
III. Bergmann's plating technique involves embedding cells in a shallow layer of agar.
IV. Single cells divide most efficiently when cultured in extremely large volumes of fresh liquid media.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I, III and IV
(C) II, III and IV
(D) I and IV

2021

**224.** Which physiological pathway is directly responsible for producing a wholly homozygous diploid plant in a single step from an anther culture without the use of chemical doubling agents?
(A) The emergence of bilateral symmetry due to the initiation of cotyledon primordia.
(B) The rapid accumulation of starch grains in the central vacuole.
(C) The shedding of the suspensor.
(D) The visible greening of the entire embryo.

2007

**225.** What is the fundamental disadvantage of using indirect somatic embryogenesis compared to direct somatic embryogenesis for clonal propagation?
(A) The intervening callus phase significantly increases the risk of somaclonal variation.
(B) It yields significantly fewer embryos per explant.
(C) It strictly requires the use of Agrobacterium.
(D) It can only be performed on monocot species.

2003

**226.** In the context of doubled haploid production, if a diploid plant $(2n = 2x = 24)$ undergoes anther culture followed by successful colchicine treatment, what is the ploidy and chromosome number of the resulting regenerated plant?
(A) Haploid, $n = 12$
(B) Diploid, $2n = 2x = 24$ (Homozygous)
(C) Tetraploid, $2n = 4x = 48$
(D) Triploid, $3n = 3x = 36$

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2007

**227.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Microspore culture is generally considered more efficient for generating solid haploids than anther culture.
Reason (R): Microspore culture eliminates the somatic tissues of the anther wall, preventing the regeneration of heterozygous diploid plants.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**228.** Sequence the physiological stages of producing a functional doubled haploid line from anthers.
I. Chromosome doubling using colchicine
II. Cold stress pretreatment of floral buds
III. Excision and culture of anthers on induction medium
IV. Regeneration of haploid plantlets from embryoids
(A) II, III, IV, I
(B) III, II, I, IV
(C) II, I, III, IV
(D) I, II, III, IV

2011

**229.** Identify the incorrect statement regarding ovary and ovule culture (gynogenesis).
(A) It is the universally preferred primary method for haploid production in all Poaceae crops.
(B) It is frequently employed when anther culture

List I: (Conservation Strategy)
P. Normal maintenance
Q. Slow-growth storage
R. Cryopreservation
S. Synthetic seeds
List II: (Defining Condition)
1. Storage in liquid nitrogen at $-196^{\circ}C$
2. Culturing at reduced temperatures with osmotic agents
3. Encapsulation of somatic embryos for short-term cold storage and direct sowing
4. Routine subculturing every 3-4 weeks at $25^{\circ}C$

(A) P-4, Q-2, R-1, S-3
(B) P-2, Q-1, R-4, S-3
(C) P-1, Q-4, R-3, S-2
(D) P-4, Q-2, R-1, S-3

**2016**

**246.** What is the primary physiological basis for maintaining a high pH (approx. 10.5) in the presence of calcium ions during protoplast fusion?
(A) It promotes rapid cell wall regeneration.
(B) It neutralizes negative charges on the plasma membrane to allow closer contact.
(C) It induces the formation of haploid embryos.
(D) It acts as an exogenous carbon source for the fused cells.

**2005**

**247.** The primary distinguishing feature of Nitsch's medium compared to MS medium is its optimization for the culture of:
(A) Anthers and microspores, often containing a specific balance of iron and varying sucrose levels.
(B) Conifer somatic embryos due to its extremely high ammonium content.
(C) Protoplasts, by completely omitting all vitamins.
(D) Hairy roots, by excluding all carbon sources.

**2021**

**248.** In micropropagation, the repeated and excessive use of high concentrations of cytokinins over multiple subcultures frequently results in:
(A) Hyperhydricity and the formation of clustered, stunted, or distorted shoots.
(B) Complete inhibition of axillary bud break.
(C) Immediate induction of taproots.
(D) Direct transition into flowering regardless of photoperiod.

**2007**

**249.** Identify the correct statement regarding artificial seed production using sodium alginate.
(A) The hardening of the bead occurs instantaneously upon contact with a calcium chloride solution via ion exchange.
(B) The concentration of sodium alginate typically ranges between 20-30% for optimal bead formation.
(C) The process is highly exothermic and severely damages the enclosed embryo.
(D) The resulting beads are completely impermeable to water and gases.

**2003**

**250.** Which physiological pathway is directly responsible for producing a wholly homozygous diploid plant in a single step from an anther culture without the use of chemical doubling agents?
(A) Symmetrical division of the microspore followed by spontaneous chromosome endoreduplication during early embryogenesis.
(B) Direct somatic embryogenesis from the anther wall septum.
(C) Meiotic reduction of a spontaneously generated tetraploid callus.
(D) Infection of the microspore by Agrobacterium tumefaciens carrying a ploidy-doubling plasmid.

**********

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2010

**237.** Which strategy involves isolating and culturing cells that survive exposure to heavy metals to generate tolerant lines?
(A) In vitro selection
(B) Cybridization
(C) Gynogenesis
(D) Thermotherapy

2008

**238.** In determining the lethal dose (LD50) of a chemical mutagen, why is it critical to establish this baseline before proceeding with large-scale mutagenesis?
(A) Doses significantly above LD50 cause excessive tissue necrosis and prevent plant regeneration.
(B) Doses below LD50 yield maximum mutations but zero survival.
(C) It determines the exact number of base pairs mutated.
(D) It proves the mutagen is actually an auxin.

2001

**239.** Which class of compounds is synthesized by plant cells in response to biotic or abiotic elicitors as a defense mechanism?
(A) Phytoalexins
(B) Auxins
(C) Primary metabolites
(D) Vitamins

2019

**240.** The Ri plasmid of Agrobacterium rhizogenes induces hairy root formation. The high growth rate of these roots in hormone-free medium is directly attributed to:
(A) Altered cellular sensitivity and synthesis of endogenous auxins governed by the transferred 'rol' genes.
(B) The endogenous synthesis of highly active cytokinins encoded by the opine genes.
(C) The physical presence of the bacteria inside the root vascular tissue.
(D) The total suppression of all secondary metabolic pathways.

2016

**241.** Sequence the essential operational phases of scaling up a plant cell suspension in a bioreactor for secondary metabolite production.
I. Inoculation of the bioreactor with the starter culture
II. Establishment of a friable callus from the source explant
III. Harvesting of the cells or secreted product
IV. Initiation of a small-scale shake-flask suspension culture
(A) II, IV, I, III
(B) IV, II, I, III
(C) II, I, IV, III
(D) I, II, IV, III

2006

**242.** Biotransformation in plant tissue culture is most appropriately defined as the:
(A) Enzymatic conversion of an exogenously supplied precursor into a high-value specific chemical product by cultured cells.
(B) Genetic engineering of a plant cell using Agrobacterium tumefaciens.
(C) Spontaneous conversion of a diploid cell into a tetraploid cell.
(D) Conversion of liquid culture into a solid callus mass.

2012

**243.** Consider the following statements concerning cryoprotectants:
I. They must be completely non-toxic at all concentrations at room temperature.
II. Glycerol acts as a penetrating cryoprotectant.
III. They lower the freezing point of the intracellular fluid.
IV. Proline is frequently used as it provides natural osmoprotection.
Which of the above statement(s) is/are correct?
(A) II, III and IV
(B) I, II and III
(C) I, III and IV
(D) I and II

2024

**244.** During the slow-freezing protocol for cryopreservation, samples are typically cooled at a precise, slow rate prior to plunging into liquid nitrogen. What is the physiological reason for this controlled cooling?
(A) To allow time for water to exit the cell as extracellular ice forms, safely dehydrating the cytoplasm.
(B) To allow maximal formation of large intracellular ice crystals.
(C) To allow DNA polymerase to replicate the genome one last time.
(D) To ensure all cryoprotectants are expelled from the cell.

2002

**245.** Match the in vitro conservation strategies (List I) with their defining conditions (List II).

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differentiate into all the cell types of a mature plant. Given the correct hormonal and nutritional environment in vitro, these cells can reorganize to form embryos or organs, eventually regenerating into a complete, fertile, and functional whole plant.

**10.** (B) Low auxin to high cytokinin ratio
The Skoog-Miller model describes the hormonal control of organogenesis in plant tissue culture. It established that the ratio of auxin to cytokinin determines the developmental fate of undifferentiated callus. Specifically, a medium with a low auxin concentration combined with a high cytokinin concentration promotes the formation of shoot buds. Conversely, high auxin and low cytokinin ratios favor the induction of roots.

**11.** (A) Dedifferentiation
Dedifferentiation is the physiological process where mature, specialized plant cells revert to an undifferentiated meristematic state. This transition allows the cells to regain the ability to divide, leading to the formation of a callus. This process is a critical prerequisite for plant regeneration in tissue culture, as it enables specialized tissues like leaf mesophyll to become competent for new developmental pathways.

**12.** (C) Rapid multiplication of shoots
Stage II of the micropropagation protocol is primarily focused on the rapid multiplication of propagules. During this phase, axillary or adventitious shoots are induced to proliferate using media enriched with cytokinins. This stage aims to achieve a high multiplication rate, ensuring the production of a large number of uniform shoots that can later be rooted and transferred to ex vitro conditions.

**13.** (D) Meristem tip culture combined with heat treatment.
Meristem tip culture is the standard technique for producing virus-free plants because the apical meristem is often devoid of systemic pathogens. Combining this with thermotherapy, where mother plants are exposed to high temperatures, further inhibits viral replication and movement. This integrated approach ensures the regeneration of healthy, pathogen-free progeny from infected parental stocks for large-scale agricultural and horticultural propagation.

**14.** (C) Supplementing the medium with antiviral agents like ribavirin.
Chemotherapy in plant tissue culture involves the use of specific chemical agents to eliminate systemic pathogens, particularly viruses, from infected tissues. Antiviral compounds like ribavirin are often added to the culture medium to inhibit viral replication during plant growth. This method is frequently used in conjunction with meristem culture to increase the efficiency of producing certified virus-free planting materials for crops.

**15.** (B) Log (exponential) phase
The growth kinetics of plant cell suspension cultures follow a sigmoid curve consisting of several distinct phases. The log or exponential phase is the period where the cells have adapted to the medium and divide at their maximum possible rate. During this stage, the cell number and biomass increase exponentially, making it the ideal time for subculturing or initiating experimental treatments.

**16.** (D) P-4, Q-2, R-1, S-3
Bergmann's plating involves embedding cells in agar for colony formation. The micro-chamber technique allows the microscopic observation of single cell division. The lag phase is the initial adaptation period to fresh medium where cells prepare for division. The decline phase occurs at the end of the culture cycle when cell death increases due to nutrient depletion or toxic byproduct accumulation.

**17.** (A) An intervening callus phase prior to embryo formation
Somatic embryogenesis can occur via two distinct pathways. Direct somatic embryogenesis involves the formation of embryos directly from the explant without an unorganized growth stage. In contrast, indirect somatic embryogenesis is characterized by the initial induction of an undifferentiated callus phase. The embryos then differentiate from the pro-embryogenic masses within the callus, which often increases the risk of genetic variations.

**18.** (C) Globular, Heart, Torpedo, Cotyledonary
The development of somatic embryos in dicotyledonous plants follows a specific morphological sequence. It begins with the globular stage, followed by the heart stage where bilateral symmetry is established. Subsequent

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# ANSWERS

**1.** (A) High concentration of potassium nitrate
Gamborg's B5 medium was specifically developed to support the growth of soybean callus and cell suspensions. It is characterized by its nitrogen composition, containing a high concentration of potassium nitrate and a relatively low amount of ammonium sulfate. This specific balance prevents the rapid pH drops often associated with higher ammonium levels found in other media formulations during plant growth.

**2.** (C) It functions as a structural component of the cell wall and aids in membrane biosynthesis.
Myo-inositol is a crucial vitamin-like supplement in plant tissue culture media, particularly in Murashige and Skoog formulations. It serves as a precursor for the biosynthesis of cell wall polysaccharides, such as pectin and hemicellulose. Additionally, it plays a vital role in phosphoinositide signaling pathways and helps in maintaining the structural integrity of cellular membranes during rapid growth.

**3.** (A) Excised tomato roots
White's medium, formulated in the late 1930s, represents one of the earliest successful chemically defined media for plant tissue culture. It was specifically optimized for the continuous growth and maintenance of excised tomato roots. This medium is characterized by a low salt concentration compared to modern formulations like MS, making it suitable for certain sensitive root cultures and physiological studies.

**4.** (D) The total ionic concentration of MS medium is significantly lower than that of White's medium.
Murashige and Skoog medium is renowned for its high concentration of mineral salts, particularly nitrate and ammonium. Unlike White's medium, which is a low-salt formulation, MS medium has a very high total ionic concentration. This high nutrient availability supports the rapid growth and morphogenesis of many plant species, making it the most widely used medium in plant biotechnology.

**5.** (B) Gibberellic acid (GA3)
During the preparation of tissue culture media, certain compounds are heat-labile and decompose under the high temperature and pressure of an autoclave. Gibberellic acid is a prime example of a thermolabile growth regulator that loses biological activity when heated. Consequently, it must be sterilized using membrane filtration and added aseptically to the basal medium after it has cooled.

**6.** (C) $121^{\circ}C$ at $15psi$ for 15-20 minutes
The autoclave is the standard instrument for sterilizing plant tissue culture media and equipment using moist heat. The most effective physical parameters to ensure the destruction of all microorganisms, including resilient bacterial endospores, are a temperature of 121 degrees Celsius and a pressure of 15 pounds per square inch. This cycle typically lasts for 15 to 20 minutes for standard volumes.

**7.** (D) It is highly toxic and requires thorough washing to prevent injury.
Mercuric chloride is a powerful surface sterilant used for cleaning plant explants, especially those from woody species. However, its significant disadvantage is its extreme toxicity to both human health and plant tissues. If the chemical is not thoroughly rinsed away with sterile distilled water after treatment, residual mercury can cause severe tissue necrosis and inhibit the growth of the explant.

**8.** (A) I and III
Sodium hypochlorite is a common surface sterilant that releases chlorine to kill microorganisms. To improve its efficacy, surfactants like Tween-20 are often added to the solution to reduce surface tension, allowing the sterilant to reach crevices and ensure uniform contact. In contrast, ethanol is used for very brief exposures, as 30 minutes would be lethal to most plant tissues.

**9.** (B) Regenerate into a complete whole plant under appropriate stimuli.
Cellular totipotency is a fundamental concept in plant biology, describing the inherent capacity of a single nucleated plant cell to divide and

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precursor. These modifications can include hydroxylation, glycosylation, or methylation. This technique is highly valuable for producing complex, stereo-specific compounds that are difficult or expensive to synthesize through traditional chemical methods, using the plant's natural metabolic machinery.

**47.** (D) Liquid nitrogen at $-196^{\circ}C$
Cryopreservation is the long-term storage of biological materials at ultra-low temperatures, where metabolic activities and cell divisions are completely arrested. This is typically achieved by immersing the samples in liquid nitrogen at -196 degrees Celsius. At this temperature, the samples can be preserved indefinitely without genetic change, provided they are treated with cryoprotectants to prevent ice crystal formation during freezing.

**48.** (A) Intracellular ice crystal formation
During cryopreservation, the most significant threat to cell survival is the formation of large, sharp intracellular ice crystals. These crystals can rupture cellular membranes and organelles, leading to irreversible cell death upon thawing. Cryoprotectants are used to lower the freezing point and increase the viscosity of the cytoplasm, encouraging the transition into a glassy state that avoids lethal crystallization.

**49.** (A) Both A and R are true and R is the correct explanation of A.
Vitrification is a cryopreservation technique that achieves a glassy, amorphous state of the cytoplasm instead of ice crystals. It involves treating tissues with highly concentrated cryoprotectant solutions that severely dehydrate the cells. When these treated tissues are rapidly plunged into liquid nitrogen, the remaining water solidifies into a glass-like state, bypassing the destructive crystalline phase and ensuring cellular survival.

**50.** (A) I, II and IV
Slow-growth germplasm conservation is used for short-to-medium term storage by reducing the metabolic rate of cultures. This is achieved by lowering the incubation temperature and adding osmotic agents like mannitol to the medium. While these methods extend the subculture interval, they do not entirely eliminate the risk of somaclonal variation, which can still accumulate over time during repeated maintenance cycles.

**51.** (A) Vitamin B1 (Thiamine)
Vitamins are essential organic compounds that function as co-factors for various enzymes in plant metabolism. Among them, Vitamin B1, or thiamine, is considered an absolute requirement in almost all plant tissue culture media formulations. It is critical for carbohydrate metabolism and the citric acid cycle. While plants can synthesize vitamins in vivo, cultured cells often require an exogenous supply to maintain growth.

**52.** (C) It efficiently translocates across cell membranes and easily breaks down into glucose and fructose.
Sucrose is the most widely used carbon and energy source in plant tissue culture. It is preferred because it is readily available, relatively inexpensive, and highly soluble. More importantly, plant cells efficiently transport sucrose across their membranes. Once inside, it is easily hydrolyzed by invertase into glucose and fructose, which enter the primary metabolic pathways to support cell division and development.

**53.** (A) P-3, Q-4, R-1, S-2
Macro-nutrients serve vital structural and physiological roles in tissue culture. Nitrogen is essential for amino acid and nucleic acid synthesis. Phosphorus is a component of ATP and nucleic acids, facilitating energy transfer. Calcium is critical for cell wall stability and membrane function. Magnesium is the central atom in the chlorophyll molecule and acts as a cofactor for many important metabolic enzymes.

**54.** (D) Precipitation of insoluble calcium salts
During the preparation of MS medium stock solutions, calcium chloride is typically kept separate from the concentrated phosphate and sulfate stocks. This prevents the formation of insoluble precipitates, such as calcium phosphate or calcium sulfate, which would settle out of the solution and make the nutrients unavailable to the plant. These components are combined only when preparing the final diluted culture medium.

**55.** (B) Absolute ethanol fails to denature proteins effectively without the presence of water.
In surface sterilization, 70% ethanol is more effective than absolute ethanol. The presence of water in the 70% solution is essential because it facilitates the penetration of ethanol into the microbial cell and aids in the efficient

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elongation leads to the torpedo stage, which finally matures into the cotyledonary stage. This progression mimics the natural development of zygotic embryos within the seed during standard plant embryogenesis and seed development.

**19.** (A) Sodium alginate and calcium chloride
Artificial seeds are produced by encapsulating somatic embryos or other propagules in a protective hydrogel. The most common encapsulation system uses sodium alginate and calcium chloride. When droplets of sodium alginate containing the embryo are dropped into a calcium chloride solution, an ion-exchange reaction occurs, resulting in the formation of a solid, protective calcium alginate bead or synthetic seed.

**20.** (D) Cultivates the intact anther containing microspores.
Anther culture and microspore culture are both techniques used to produce haploid plants, but they differ in the starting material. Anther culture involves the cultivation of the entire intact anther, where the surrounding somatic tissues can influence development. In microspore culture, the pollen grains are isolated and cultured away from the anther wall, providing a more controlled system for androgenesis.

**21.** (B) The microspore divides symmetrically into identical cells.
In pollen embryogenesis, Pathway I is characterized by a symmetrical division of the microspore. Unlike the normal asymmetrical division that produces a large vegetative cell and a small generative cell, this symmetrical division results in two identical nuclei. These identical cells then continue to divide, bypassing the gametophytic pathway and leading directly to the formation of a haploid somatic embryo.

**22.** (C) By preventing the formation of spindle fibers during mitosis
Colchicine is an alkaloid used in plant breeding to induce polyploidy or double the chromosome number of haploids. It works by binding to tubulin dimers, which prevents the polymerization of microtubules into spindle fibers during mitosis. Consequently, the chromosomes replicate and separate but are not pulled to opposite poles, resulting in a single cell with a doubled set of chromosomes.

**23.** (D) Unfertilized ovaries or ovules
Gynogenesis is an in vitro technique used to produce haploid plants from the female gametophyte. It involves the culture of unfertilized ovaries or isolated ovules. The haploid cells within the embryo sac, such as the egg cell or synergids, are induced to divide and form embryos or callus. This method is particularly useful for species where anther culture is unsuccessful.

**24.** (A) Production of haploids in male-sterile crop species.
Gynogenesis provides a valuable alternative to androgenesis, especially when dealing with male-sterile plant lines where viable pollen is unavailable. It allows breeders to generate haploids from the female gametic tissues of these plants. Additionally, gynogenesis is often preferred in certain species, like those in the Poaceae family, where anther culture frequently results in a high frequency of undesirable albino plants.

**25.** (A) Both A and R are true and R is the correct explanation of A.
Interspecific hybridization often results in post-zygotic barriers where the hybrid embryo fails to develop properly due to endosperm collapse. Embryo rescue addresses this by excising the immature embryo before it aborts and culturing it on a nutrient-rich medium. This technique bypasses the nutritional deficiency caused by endosperm failure, allowing for the successful recovery and growth of distant hybrid progeny.

**26.** (B) Failure of the pollen tube to reach the stigma.
Embryo rescue is an effective technique for overcoming post-zygotic barriers, such as endosperm degeneration or spontaneous embryo abortion. However, it cannot mitigate pre-zygotic barriers, such as the failure of the pollen tube to reach the stigma or enter the ovule. These earlier incompatibility issues must be addressed through other methods, such as in vitro pollination or style cutting techniques.

**27.** (A) Triploid plants
Endosperm culture is a specialized tissue culture technique used to produce triploid plants directly. In most angiosperms, the endosperm is a triploid tissue formed by the fusion of two polar nuclei and one male gamete. By excising and culturing this tissue, researchers can regenerate plants

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suffers from high frequencies of albinism.
(C) The responding cell is typically the unfertilized egg cell or synergids.
(D) Excision of ovaries is generally less complex than isolating individual ovules.

2016

**230.** During embryo rescue in intergeneric crosses, if an embryo is excised too early (globular stage), it:
(A) Strictly requires a highly complex medium often supplemented with specific amino acids.
(B) Is highly robust and grows rapidly on basal media.
(C) Spontaneously undergoes polyploidization.
(D) Directly converts into endosperm tissue.

2006

**231.** The successful generation of a triploid plant from endosperm culture typically requires the continuous presence of:
(A) A combination of auxin and cytokinin
(B) Gibberellic acid alone
(C) High levels of colchicine
(D) Agrobacterium rhizogenes

2012

**232.** Match the specialized culture techniques (List I) with their defining primary application (List II).
List I: (Enzyme)
P. Cellulase Onozuka
Q. Macerozyme
R. Pectolyase
S. Driselase
List II: (Commercial Source)
1. Trichoderma viride
2. Rhizopus spp.
3. Aspergillus japonicus
4. Basidiomycete fungi
(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-1, R-4, S-3
(C) P-3, Q-4, R-1, S-2
(D) P-1, Q-3, R-2, S-4

2016

**233.** During protoplast viability testing with FDA, why does the green fluorescence eventually fade if the protoplasts are left under the UV microscope for too long?
(A) Fluorescein leaks out of the cell because the plasma membrane loses integrity, and the dye is subject to photobleaching.
(B) The intracellular esterases rapidly denature FDA into a non-fluorescent black compound.
(C) The UV light permanently fuses the protoplasts together.
(D) The cell wall suddenly regenerates and blocks the light.

2006

**234.** Consider the following statements concerning cybridization:
I. It allows the transfer of organelle-encoded traits like CMS.
II. It requires the physical elimination or inactivation of one parental nucleus.
III. It always results in the mixing of the nuclear genomes of both parents.
IV. Nuclear inactivation is commonly achieved using irradiation.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) II, III and IV
(C) I, II and III
(D) I and IV

2024

**235.** The formation of a homokaryon during protoplast fusion is defined as:
(A) The fusion of two protoplasts originating from the same parental species or line.
(B) The fusion of a protoplast with an enucleated cytoplast.
(C) The fusion of a protoplast with a fungal cell.
(D) The fusion of a somatic cell with a gametic cell.

2003

**236.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
Assertion (A): Transposon activation during the tissue culture process is a significant source of genetic somaclonal variation.
Reason (R): The in vitro culture environment induces severe cellular stress, which can cause previously silenced transposable elements to excise and reinsert randomly throughout the genome.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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**37.** (A) Any form of somatic cell or tissue culture.
Somaclonal variation describes the genetic and epigenetic changes that occur in plants regenerated from any type of in vitro somatic cell or tissue culture. These variations can arise due to chromosomal rearrangements, point mutations, or DNA methylation shifts. While often considered a problem for maintaining clonal fidelity, somaclonal variation can also be a source of novel traits for plant improvement.

**38.** (C) Altered DNA methylation patterns.
Epigenetic somaclonal variation refers to changes in gene expression that do not involve alterations to the underlying DNA sequence. A primary mechanism for this is the alteration of DNA methylation patterns, where the addition or removal of methyl groups affects whether genes are active or silenced. These changes are often reversible but can be inherited through several generations of regenerated plants.

**39.** (A) Sodium chloride (NaCl)
In vitro selection for salinity tolerance involves exposing cell cultures to increasing concentrations of salt. Sodium chloride is the appropriate selective agent added to the culture medium for this purpose. Cells that can survive and proliferate under high salt stress are isolated and regenerated into plants. This approach aims to identify mutants with improved physiological mechanisms for managing osmotic and ionic stress.

**40.** (B) I, II and III
Chemical mutagens like EMS and physical mutagens like gamma rays are effective tools for inducing genetic variation in vitro. The LD50 value is a critical parameter representing the dose that kills half of the population, helping to balance mutation frequency with survival. While mutations can be screened in the M1 generation, they are often more reliably characterized in subsequent generations.

**41.** (A) Ultraviolet (UV) radiation
Elicitors are substances or treatments that trigger the synthesis of secondary metabolites in plant cell cultures as a defense response. Abiotic elicitors are non-biological factors, such as ultraviolet radiation, heavy metal salts, or thermal stress. These physical or chemical stimuli induce physiological stress in the cells, activating metabolic pathways that lead to the accumulation of protective compounds like alkaloids or phenolics.

**42.** (B) Agrobacterium rhizogenes
Hairy root cultures are induced by infecting plant tissues with the soil-borne bacterium Agrobacterium rhizogenes. This bacterium transfers a specific segment of its root-inducing plasmid into the plant genome. The resulting transgenic roots are characterized by rapid, highly branched growth on hormone-free media. They are genetically stable and often produce high levels of secondary metabolites similar to the original plant's roots.

**43.** (D) rol genes (rol A, B, C, D)
The formation of hairy roots is driven by the integration and expression of 'rol' genes from the Ri plasmid of Agrobacterium rhizogenes. These genes, specifically rolA, rolB, rolC, and rolD, modulate the plant's hormonal balance and sensitivity to auxins. Their expression triggers the neoplastic development of roots at the site of infection, which then exhibit the characteristic fast-growing hairy root phenotype.

**44.** (D) Airlift bioreactor
Airlift bioreactors are designed to provide efficient mixing and aeration without the use of mechanical impellers. Instead, they use a stream of air to circulate the liquid medium through an internal or external loop. This design significantly reduces hydrodynamic shear stress, making it ideal for fragile plant cell suspension cultures that are easily damaged by the mechanical agitation of stirred-tank bioreactors.

**45.** (B) Calcium alginate beads
Immobilization of plant cells involves entrapping them in a semi-solid matrix to facilitate continuous production of metabolites. Calcium alginate beads are the most common matrix used for this purpose. The beads are formed through an ion-exchange process between sodium alginate and calcium chloride. This matrix is non-toxic, provides physical protection to the cells, and allows for the easy recovery of secreted products.

**46.** (C) Cells modify an exogenous precursor into a valuable product.
Biotransformation is a process where cultured plant cells utilize their endogenous enzyme systems to catalyze specific chemical modifications on an exogenously supplied

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that possess three sets of chromosomes, which is highly valuable for developing seedless fruit varieties.

**28.** (B) It results in seedless fruits due to meiotic sterility.
Triploid plants produced via endosperm culture are highly valued in horticultural breeding because they are generally seedless. The presence of three sets of chromosomes leads to irregular meiosis and the formation of non-viable gametes, resulting in sterility. This trait is commercially desirable in fruits like watermelons, citrus, and grapes, where consumers prefer the absence of seeds for consumption.

**29.** (A) I and III
Statement I is incorrect because cellulases target the cellulose in the cell wall, while pectinases or macerozymes are required to degrade the pectin middle lamella. Statement III is also incorrect because osmotic stabilizers are absolutely essential during enzymatic digestion to prevent the immediate lysis of the fragile, wall-less protoplasts. Osmotica must be maintained to balance the internal cellular pressure.

**30.** (B) To act as osmotic stabilizers to prevent protoplast lysis.
When the rigid plant cell wall is enzymatically removed, the resulting protoplast becomes extremely sensitive to osmotic changes. Mannitol and sorbitol are non-metabolizable sugar alcohols added to the enzyme solution to act as osmotic stabilizers. They maintain an iso-osmotic or slightly hypertonic environment, preventing the influx of water that would otherwise cause the protoplasts to swell and burst.

**31.** (A) FDA is converted to fluorescein by intracellular esterases.
Fluorescein diacetate is a non-fluorescent, non-polar molecule that can freely diffuse across the plasma membrane of living cells. Once inside a viable protoplast, intracellular esterases cleave the acetate groups, releasing polar fluorescein. Fluorescein is fluorescent and cannot easily exit the cell, causing viable protoplasts to glow green under UV light, which serves as a reliable indicator of metabolic activity.

**32.** (B) Polyethylene glycol (PEG)
Polyethylene glycol is the most widely used chemical fusogen for inducing protoplast fusion. It acts by inducing membrane agglutination and bringing adjacent protoplasts into close contact. When the PEG is subsequently eluted with a high pH and high calcium solution, the membranes fuse. PEG is preferred due to its high fusion efficiency and relatively low toxicity across a wide variety of plant species.

**33.** (D) III, IV, I, II
Electrofusion begins with the application of a low-voltage alternating current field, which causes protoplasts to align into "pearl chains" via dielectrophoresis. Once aligned, a short, high-voltage direct current pulse is applied to create reversible pores in the plasma membranes at the points of contact. This membrane breakdown leads to the subsequent fusion of the adjacent protoplasts into a single hybrid cell.

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**34.** (C) It has one parental nucleus and the other parent's cytoplasm.
A cybrid, or cytoplasmic hybrid, is a cell that contains the nuclear genome of only one parent but the cytoplasm and organelles from both parents, or just the other parent. This differs from a symmetric somatic hybrid, which contains the full nuclear genomes of both parental species. Cybridization is useful for transferring organelle-encoded traits, such as cytoplasmic male sterility, between lines.

**35.** (A) Gamma irradiation of the donor protoplast to inactivate its nucleus.
The donor-recipient method is a strategy used in cybridization to achieve organelle transfer. It involves treating the donor protoplast with a high dose of gamma radiation to completely inactivate or fragment its nucleus. This irradiated donor is then fused with a recipient protoplast. The resulting hybrid retains the recipient's nucleus but incorporates the donor's cytoplasm and organelles, effectively creating a cybrid.

**36.** (D) Mitochondrial genome
Cytoplasmic male sterility is a valuable trait in hybrid seed production that prevents the formation of functional pollen. The genetic determinants for CMS are located within the mitochondrial genome rather than the nucleus. Because mitochondria are inherited through the cytoplasm, cybridization is an effective method for transferring CMS from a donor species into a desirable recipient line by merging their cytoplasms.

denaturation of proteins. Absolute ethanol tends to dehydrate the cell surface rapidly, creating a protective layer that can prevent the alcohol from killing the organism.

**56.** (B) It destroys vegetative cells but is ineffective against bacterial endospores.
Autoclaving is a highly effective method of sterilization because it uses saturated steam under high pressure to achieve temperatures above the boiling point of water. This process is specifically designed to destroy all forms of life, including highly resilient bacterial endospores. Standard sterilization cycles of 121 degrees Celsius for 15 to 20 minutes are sufficient to ensure that the media is completely sterile.

**57.** (A) Meristematic callus cells mature and specialize into distinct plant organs.
Redifferentiation is the phase in tissue culture where undifferentiated callus cells respond to specific hormonal signals by specializing into distinct tissues or organs, such as shoots or roots. This process is the reverse of dedifferentiation and represents the final stage of plant regeneration. It demonstrates the ability of plant cells to reorganize and express the genetic information required to form complex structures.

**58.** (C) Stage II - Hardening and acclimatization of plantlets
In the micropropagation protocol, Stage II is dedicated to the rapid multiplication of shoots through the induction of axillary or adventitious buds. The hardening and acclimatization of plantlets actually occur in Stage IV, which is the final phase before the plants are moved to the field. This stage involves the gradual adaptation of the in vitro-grown plants to environmental conditions.

**59.** (B) Viral pathogens
Thermotherapy involves exposing mother plants or in vitro cultures to elevated temperatures, typically between 35 and 40 degrees Celsius, for several weeks. This treatment is specifically used to eliminate systemic viral pathogens. High temperatures inhibit the replication and movement of viruses within the plant tissues, allowing the healthy meristem to grow away from the infected areas, which can then be excised.

**60.** (C) Constant rate of cell division coupled with steady cell expansion
During the linear phase of cell suspension culture growth, the initial exponential growth rate begins to slow down. However, the culture still exhibits a constant and steady increase in biomass. This phase is characterized by a balance where the rate of cell division is relatively stable, and cells continue to expand in size, reflecting a sustained period of active primary metabolism.

**61.** (A) Cells are mixed with warm, liquid agar medium and spread in a thin layer in a Petri dish.
Bergmann's plating technique is a fundamental method for single-cell culture. It involves suspending isolated cells in a warm, liquid medium containing a low concentration of agar. This mixture is then poured into a Petri dish to form a thin, solid layer. This spatial separation allows individual cells to divide and form isolated colonies, which can be easily identified and subcultured.

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**62.** (B) Heart stage
In the development of dicot somatic embryos, the heart stage is a pivotal transition point. It is characterized by the initiation of two cotyledon primordia, which gives the embryo its distinctive heart-like shape. This stage marks the shift from radial to bilateral symmetry, indicating that the apical-basal axis and the basic body plan of the future plant have been successfully established.

**63.** (B) I, III and IV
Somatic embryogenesis is the process of forming embryos from somatic cells rather than gametic fusion. These embryos are bipolar, having both a shoot and a root pole, and they lack a direct vascular connection with the maternal tissue. High concentrations of the synthetic auxin 2,4-D are commonly used to induce this process in a wide variety of plant species.

**64.** (A) II, IV, I, III
The encapsulation-dehydration protocol for cryopreservation follows a logical sequence to ensure cell survival. First, the explants are encapsulated in calcium alginate beads for protection. They are then pre-cultured in high-sucrose medium to increase internal osmotic concentration. Next, air desiccation is used to remove excess water. Finally, the beads are

distilled water, they can penetrate the plant tissues. Residual sterilants cause phytotoxicity, leading to tissue browning, necrosis, and the eventual death of the explant, which prevents the successful initiation of a healthy aseptic culture.

**120.** (A) Auxin is significantly higher than cytokinin.
According to the Skoog-Miller model, the differentiation of tissues in a callus is controlled by the balance of growth regulators. Root organogenesis is specifically promoted when the culture medium contains a relatively high concentration of auxin and a low concentration of cytokinin. This hormonal ratio signals the undifferentiated cells to organize into a root apical meristem, initiating the formation of adventitious roots.

**121.** (A) Totipotent cells have the capacity to regenerate into a complete, fertile plant, whereas pluripotent cells can only differentiate into specific tissue lineages.
Cellular totipotency is the unique ability of a plant cell to regenerate an entire organism, including all reproductive structures. This is a higher level of competence than pluripotency, which describes cells that can form various tissue types but cannot organize themselves into a whole plant. In tissue culture, the goal is often to exploit totipotency to regenerate complete plants from single cells or tissues.

**122.** (A) Complete elimination of viral pathogens.
The size of the explant in meristem culture is a critical factor for the production of virus-free plants. Viruses are typically absent from the very tip of the apical dome. By excising an extremely small portion, usually less than 0.5 mm, the chance of including viral particles is minimized. This ensures that the regenerated plantlet starts its life completely free from systemic viral infections.

**123.** (D) It relies on liquid nitrogen to shock-kill systemic viruses.
Thermotherapy is a technique that uses heat, not cold, to eliminate viruses. By exposing infected mother plants to temperatures between 35 and 40 degrees Celsius for an extended period, the replication and movement of viruses are suppressed. This treatment is often used in combination with meristem culture, as the high heat helps to expand the virus-free zone at the shoot tip.

**124.** (B) A net zero growth rate where cell division equals cell death, often due to nutrient depletion.
The stationary phase is the point in a cell suspension culture where the increase in biomass stops. This occurs because the rate of new cell division is balanced by the rate of cell death. The cessation of growth is typically caused by the depletion of essential nutrients in the medium or the accumulation of toxic metabolic byproducts, indicating that the culture needs subculturing.

**125.** (B) Isolating a single cell in a small droplet of medium on a specialized slide to observe continuous division.
The micro-chamber technique is a specialized method used to study the growth of individual plant cells. A single cell is isolated in a very small volume of nutrient medium and placed on a slide, often with a "nurse" tissue or conditioned medium to provide growth factors. This setup allows researchers to monitor the division and development of a single cell under a microscope.

**126.** (A) I, II and IV
Synthetic seeds are produced by encapsulating somatic embryos in a protective hydrogel like calcium alginate. This matrix provides a physical barrier and can be supplemented with nutrients and pesticides. While they can be handled like natural seeds, they are produced from somatic tissues rather than zygotic embryos. This technology allows for the direct field delivery of elite, clonally propagated plant materials.

**127.** (A) Suspensor
The suspensor is a vital embryonic structure that provides both structural and nutritional support to the developing embryo. It anchors the embryo to the maternal tissue and facilitates the transfer of nutrients from the endosperm. In embryo culture, the presence of an intact suspensor is often critical for the successful survival and development of very young embryos, mimicking its essential role during natural seed development.

**128.** (A) All three pathways can yield haploid embryos depending on the species.
Androgenesis can occur through several distinct pathways within the microspore. Pathway I involves symmetrical division, while Pathways II and III involve asymmetrical division followed by the division of the vegetative or generative cell, respectively. Although the specific route depends

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**74.** (B) Using complementary biochemical mutants (auxotrophs)
After protoplast fusion, identifying the hybrid cells from the parental population is essential. One effective strategy uses complementary auxotrophic mutants, where each parent is unable to grow on a basic medium due to a specific nutritional deficiency. The hybrid cell, however, inherits functional genes for both pathways from the opposite parents, allowing it to grow and be selected on the unsupplemented medium.

**75.** (B) Generating novel, unpredicted phenotypic variations is desired.
While somaclonal variation is undesirable for maintaining uniform clones, it is a valuable tool for plant breeders looking for new traits. The variations induced during tissue culture can lead to plants with improved characteristics, such as disease resistance, salt tolerance, or altered growth habits. This provides a source of novel genetic diversity that can be integrated into traditional breeding programs.

**76.** (C) Gametic cells such as microspores
Gametoclonal variation specifically refers to the genetic and epigenetic variations observed in plants regenerated from gametic tissues, such as microspores in anther culture or egg cells in ovary culture. Because these variations originate from haploid cells, they can be fixed into a homozygous state more rapidly than variations arising in diploid somatic tissues, offering a unique tool for genetic studies.

**77.** (C) DNA methylation shifts affecting gene expression
Epigenetic variation involves changes in gene function that do not alter the DNA sequence itself. In tissue culture, shifts in DNA methylation are a primary mechanism for this. These changes can silence or activate genes, leading to phenotypic variations in regenerated plants. Unlike genetic mutations, epigenetic changes are often unstable and may revert in subsequent generations or during the sexual cycle.

**78.** (B) They require the constant exogenous addition of auxins for growth.
Hairy root cultures, induced by Agrobacterium rhizogenes, are unique because they grow vigorously on culture media without any exogenous growth regulators. The integration of 'rol' genes from the bacterium increases the sensitivity of the roots to endogenous auxins, allowing them to maintain rapid, highly branched growth autonomously. This hormonal independence is a defining characteristic of the hairy root phenotype.

**79.** (B) Sparging gas from the bottom of the vessel
In a bubble column bioreactor, mixing and aeration of the cell suspension are achieved solely by sparging air or other gases from the bottom of the vessel. The rising bubbles create turbulence and move the liquid, ensuring that the cells remain suspended and have access to oxygen. This design avoids mechanical agitation, which helps protect shear-sensitive plant cells from physical damage.

**80.** (A) I
Biotic elicitors, such as fungal cell wall fragments or yeast extract, mimic a pathogen attack when added to plant cell cultures. This triggers a defense response characterized by the activation of the hypersensitive response and the rapid accumulation of phytoalexins and other secondary metabolites. This strategy is widely used in biotechnology to significantly increase the yields of valuable plant-derived compounds.

**81.** (B) Adding an intermediate compound to the medium for specific structural modification by the cells.
Precursor feeding is a biotransformation strategy where an intermediate compound from a metabolic pathway is added to the culture medium. The plant cells then take up this precursor and use their endogenous enzymes to convert it into the desired final product. This approach bypasses rate-limiting steps in the biosynthetic pathway, leading to a higher concentration of the target secondary metabolite.

**82.** (A) They lower the freezing point and minimize the formation of large, lethal intracellular ice crystals.
Cryoprotectants like dimethyl sulfoxide play a vital role in cryopreservation by protecting cells from freezing injury. They penetrate the cell membrane and interact with water molecules to lower the freezing point and increase the viscosity of the cytoplasm. This helps to prevent the formation of large, sharp ice crystals that would otherwise rupture the plasma membrane and cause irreversible damage during freezing.

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plasma membrane. Living, viable cells have an intact membrane that prevents the dye from entering the cytoplasm. In contrast, dead or damaged cells have permeable membranes that allow the blue dye to accumulate, making them easily distinguishable under a microscope.

**102.** (A) II and IV
Statement II is incorrect because successful chemical fusion using calcium ions typically requires a high pH (around 10.5) rather than a low pH. Statement IV is also incorrect as chemical fusion using PEG is not strictly species-specific; it is widely used to create interspecific and even intergeneric somatic hybrids between sexually incompatible plants, making it a versatile tool for wide hybridization.

**103.** (A) Retains the full nuclear genome of both parental protoplasts.
A symmetric somatic hybrid is formed by the complete fusion of two intact protoplasts from different parents. The resulting hybrid cell and the plant regenerated from it contain the full complement of nuclear chromosomes from both parental species. This type of hybrid allows for the combination of all nuclear traits, although it often requires subsequent breeding to manage the doubled chromosome number.

**104.** (A) Both A and R are true and R is the correct explanation of A.
Cybridization is an effective method for transferring cytoplasmic male sterility because CMS is encoded by genes in the mitochondria. During the formation of a cybrid, the cytoplasm of two parents is mixed, allowing the donor's mitochondria to be incorporated into the recipient's cell. Because the donor's nucleus is inactivated, the resulting plant retains the recipient's nuclear traits while gaining the donor's CMS.

**105.** (A) Prolonged undifferentiated callus phase
Point mutations and other forms of somaclonal variation are most likely to occur during an extended period of undifferentiated growth, such as in a long-term callus or cell suspension culture. The rapid and unorganized cell division under stressful in vitro conditions can lead to errors in DNA replication and repair. Consequently, minimizing the duration of the callus phase helps maintain genetic stability.

**106.** (B) Phytotoxins isolated from the target pathogen
In vitro selection for disease resistance involves exposing cell cultures to a selective agent that mimics the pathogen's effect. Often, purified phytotoxins produced by the pathogen are added to the culture medium. Only the cells with inherent resistance or those that develop mutations allowing them to survive the toxin will grow. These resistant cells are then regenerated into plants with potential disease resistance.

**107.** (A) Results in a 50% reduction in explant survival or cell viability.
The LD50 value is a critical measurement used in mutagenesis experiments. It represents the "lethal dose" that causes a 50% reduction in the survival of the treated explants or cells. Establishing this value helps researchers choose an optimal mutagen dose that is high enough to induce a significant number of mutations but low enough to allow a sufficient number of cells to survive.

**108.** (A) Auxins
The 'rol' genes transferred by Agrobacterium rhizogenes play a central role in the induction of hairy roots by modulating the plant's sensitivity to auxins. Specifically, these genes alter the cellular perception and metabolism of endogenous auxins, making the transformed cells highly responsive to low hormone levels. This increased sensitivity triggers the rapid and characteristic neoplastic root growth without the need for exogenous growth regulators.

**109.** (B) They induce physiological stress, triggering defense pathways and secondary metabolite synthesis.
Abiotic elicitors, such as heavy metal salts or UV light, function by creating a controlled level of physiological stress in plant cell cultures. This stress mimics environmental challenges, activating the plant's innate defense signaling pathways. As a result, the cells increase the production of various secondary metabolites, such as phenolics or alkaloids, which are part of the plant's natural defense mechanism.

**110.** (A) Airlift bioreactor
An airlift bioreactor is a type of vessel that achieves mixing and aeration using air sparging. It features a central draft tube or a baffle that creates two distinct zones: a riser where gas-rich

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**92.** (C) Torpedo stage - Elongation of the embryonic axis
During somatic embryogenesis in dicots, the torpedo stage follows the heart stage and is characterized by the significant elongation of the embryonic axis. This stage also sees the continued development of the cotyledons. The elongated shape of the embryo at this point resembles a torpedo, and it marks the further maturation of the vascular tissues and the shoot and root apical meristems.

**93.** (A) III, I, IV, II
The growth of a plant cell suspension culture begins with a lag phase, during which cells adapt to the new medium. This is followed by the log phase, characterized by exponential cell division. The growth then transitions into a linear phase where biomass increases at a constant rate. Finally, the culture enters the stationary phase as nutrients become limiting and growth ceases.

**94.** (A) I and III
For successful anther culture, the developmental stage of the microspores is critical, with the late uninucleate to early binucleate stage being the most responsive. Pre-treatments, such as cold stress, are often used to enhance the androgenic response by redirecting the microspores toward embryogenesis. This process ultimately leads to the formation of either haploid somatic embryos or an androgenic callus.

**95.** (B) Tubulin dimers preventing microtubule polymerization
Colchicine is a powerful polyploidizing agent that specifically binds to tubulin dimers. This binding prevents the assembly of microtubules, which are the essential building blocks of the spindle fibers required for chromosome separation during mitosis. By disrupting the spindle apparatus, colchicine ensures that the replicated chromosomes remain within a single cell, resulting in a doubling of the plant's chromosome number.

**96.** (D) The rate of cell division in the meristem exceeds the rate of viral replication and vascular connection is absent.
Meristem tip culture is effective for producing virus-free plants because the apical meristem often remains free of viruses. This is due to the rapid rate of cell division in the meristem, which often outpaces viral replication. Furthermore, the absence of a fully developed vascular system in the meristem prevents the systemic movement of viruses into the newly formed cells at the tip.

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**97.** (A) Mitigating post-zygotic barriers in distant crosses
Embryo rescue is a vital technique used in plant breeding to recover hybrid plants from distant crosses that would otherwise fail. It is specifically designed to overcome post-zygotic barriers, such as the collapse of the endosperm, which would lead to the starvation and death of the developing embryo. By culturing the embryo in vitro, breeders can successfully produce wide-hybrid progeny.

**98.** (D) Excising the developing embryo and nourishing it on an artificial nutrient medium.
In many interspecific crosses, the endosperm fails to develop correctly, which prevents the hybrid embryo from receiving the nutrients it needs to survive. Embryo rescue bypasses this problem by physically removing the immature embryo from the developing seed and placing it directly onto a nutrient-rich artificial medium. This provides the embryo with the necessary sustenance to complete its development and germinate.

**99.** (B) Endosperm
The production of triploid plants is achieved through the culture of endosperm tissue. In most flowering plants, the endosperm is naturally triploid as a result of double fertilization. When this tissue is excised and induced to regenerate in vitro, the resulting plants maintain this triploid state. Triploid plants are often seedless and are highly desirable in various horticultural fruit crops.

**100.** (A) P-2, Q-1, R-3, S-4
Protoplast isolation requires specific enzymes to target different cell wall components. Cellulase is used to digest the cellulose microfibrils in the primary wall. Pectinase specifically targets the pectin in the middle lamella that holds cells together. Hemicellulases are used to break down the matrix polysaccharides, while lipases could be used to target lipids if necessary for specific membrane-related studies.

**101.** (B) Non-viable cells accumulate the blue dye.
The Evans Blue exclusion test is a simple method for assessing the viability of isolated protoplasts. It relies on the integrity of the

**83.** (A) I
Statement I is incorrect because the primary goal of slow-growth germplasm conservation is to reduce the metabolic rate, which is typically achieved by lowering the incubation temperature significantly below the standard 25 degrees Celsius. By maintaining cultures at temperatures between 4 and 15 degrees Celsius, the rate of growth is retarded, thereby extending the time required between successive subculturing events.

**84.** (B) High risk of somaclonal variation accumulating over repeated subcultures
While slow-growth storage is a useful conservation method, its major limitation compared to cryopreservation is the risk of accumulating somaclonal variation. Because the cells are still metabolically active and continue to divide, albeit slowly, genetic and epigenetic changes can occur over time. In cryopreservation, all metabolic activity is halted, ensuring that the genetic integrity of the germplasm is preserved indefinitely.

**85.** (C) Nitrate and ammonium salts
The MS medium formulation is distinguished by its very high concentrations of nitrogen sources, particularly nitrate and ammonium. This high nutrient level supports the rapid biomass accumulation and vigorous growth of many plant species in vitro. The specific balance of these ions also helps to stabilize the pH of the medium during culture, providing an optimal environment for cell division and development.

**86.** (C) Thiamine (Vitamin B1)
Thiamine is an essential vitamin in plant tissue culture because it acts as a co-factor for enzymes involved in the citric acid cycle and carbohydrate metabolism. While plants can synthesize thiamine in vivo, isolated cells and tissues often lose this ability or cannot produce it in sufficient quantities. Therefore, thiamine is an absolute requirement in almost all medium formulations to ensure growth.

**87.** (B) To enhance contact between the sterilant and the plant surface by reducing surface tension
Surfactants, or wetting agents, are frequently added to surface sterilization solutions to improve their effectiveness. By reducing the surface tension of the liquid, the surfactant allows the sterilant to spread more evenly over the plant surface and penetrate small crevices or hairy areas. This ensures that the entire surface of the explant is in contact with the sterilizing agent, reducing contamination.

**88.** (A) Both A and R are true and R is the correct explanation of A.
Gibberellic acid is a heat-sensitive growth regulator that undergoes chemical degradation when subjected to the high temperatures and pressure of an autoclave. To maintain its biological activity, GA3 must be dissolved in a suitable solvent, sterilized by passing it through a fine membrane filter, and then added aseptically to the basal culture medium after it has been autoclaved and cooled.

**89.** (A) The loss of the requirement for exogenous growth regulators by a previously dependent culture.
Habituation is a phenomenon where plant tissue cultures, after prolonged periods of growth on media supplemented with hormones, spontaneously lose their requirement for exogenous auxins or cytokinins. These habituated cultures can then grow on basal media without any growth regulators. This change is typically considered an epigenetic event where the cells have internally activated their own hormone biosynthetic pathways.

**90.** (A) Shoot bud induction
According to the principles of organogenesis established by Skoog and Miller, the relative concentrations of auxin and cytokinin control the development of callus. A high concentration of cytokinin, such as BAP, paired with a very low concentration of an auxin like NAA, typically induces the formation of shoot buds. This hormonal balance directs the undifferentiated cells to organize into a shoot apical meristem.

**91.** (A) Selection, maintenance, and pre-treatment of the mother plant
Stage 0 is the preparatory phase of micropropagation, focusing on the selection and care of the donor or mother plant. Proper maintenance and pre-treatment of the mother plant are essential to ensure that the explants are healthy and have a low level of initial contamination. This stage significantly influences the success of subsequent steps, including the establishment of aseptic cultures and multiplication.

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liquid moves up and a downcomer where degassed liquid moves down. This cyclic flow ensures efficient nutrient and oxygen distribution while minimizing the shear stress on sensitive cells.

**111.** (A) It allows for the continuous recovery of secreted secondary metabolites without destroying the biomass.
Cell immobilization in calcium alginate beads is advantageous for biotechnology because it spatially separates the plant cells from the liquid medium. This setup allows the medium to be continuously or periodically replaced, enabling the recovery of secreted metabolites without damaging the cells. It also protects the biomass from shear stress and facilitates the long-term use of the cells in a bioreactor system.

**112.** (A) I, II and III
Vitrification is a cryopreservation method that avoids intracellular ice formation by transforming the cytoplasm into a glassy, amorphous state. This is achieved by treating cells with highly concentrated cryoprotectants and using extremely rapid cooling rates. By bypassing the crystallization phase, vitrification prevents the mechanical damage caused by ice crystals, ensuring high recovery rates for various plant tissues, including shoot tips and embryos.

**113.** (A) It acts as a non-metabolizable osmotic agent, restricting water uptake and reducing growth rate.
In slow-growth germplasm conservation, mannitol is used as an osmotic retardant. Because it is not readily metabolized by plant cells, it increases the osmotic pressure of the medium. This reduces the cell's ability to take up water, which naturally slows down the rate of cell division and expansion. This physiological slowing extends the time between subcultures, facilitating easier medium-term conservation.

**114.** (C) Stage IV (Acclimatization)
Stage IV of micropropagation is the most critical phase because plantlets transition from a controlled, high-humidity in vitro environment to the variable conditions of the greenhouse or field. During this time, the plants are highly vulnerable to desiccation because their waxy cuticles and stomatal control are not fully developed. They are also susceptible to microbial attacks as they move to non-sterile soil environments.

**115.** (B) Anthers and microspores
Nitsch's medium was specifically formulated for the in vitro culture of anthers and microspores, particularly for species within the Solanaceae family. It contains a unique balance of mineral salts and vitamins optimized to support androgenesis and the development of haploid plants. This medium has been instrumental in the production of doubled haploids for plant breeding and genetic research in various crop species.

**116.** (A) P-2, Q-4, R-1, S-3
Thiamine is an essential co-enzyme for carbohydrate metabolism, while myo-inositol is vital for membrane synthesis. Ascorbic acid acts as an antioxidant to prevent the oxidation of phenolic compounds, which causes tissue browning. Glycine is an amino acid frequently added to support growth. Together, these supplements optimize the physiological environment of the culture media, ensuring healthy cell division and plant development.

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**117.** (A) Lactose
While many sugars can serve as carbon sources in tissue culture, lactose is generally the least effective for most plant species. Most plant cells lack the specific enzymes required to efficiently transport and hydrolyze lactose into its component sugars, glucose and galactose. Consequently, it does not support vigorous growth and is rarely used compared to sucrose, glucose, or fructose in standard medium formulations.

**118.** (A) Potassium is crucial for osmotic regulation but is not a structural component of any major biomolecule.
Potassium is a vital macronutrient in plant tissue culture, primarily functioning as a major osmoticum and an activator for numerous enzymes. Unlike nitrogen, phosphorus, or sulfur, potassium is not incorporated into the structure of major biomolecules like proteins or DNA. Instead, it remains in its ionic form within the cell, where it is essential for maintaining turgor pressure and electrical balance.

**119.** (A) Both A and R are true and R is the correct explanation of A.
Surface sterilants like sodium hypochlorite are toxic chemicals used to kill surface-borne microorganisms. If these substances are not completely removed by multiple rinses with sterile

of one parent before fusion. Cybridization is particularly useful for transferring traits carried by mitochondria or chloroplasts, such as herbicide resistance or cytoplasmic male sterility, across different plant lines.

**138.** (C) It is often reversible and involves modifications such as DNA methylation or histone acetylation.
Epigenetic somaclonal variation involves changes in gene expression that do not alter the DNA sequence. These changes are often caused by chemical modifications like DNA methylation or changes to histone proteins. Because these modifications are not permanent changes to the genetic code, they can sometimes be reversed over time or during sexual reproduction, distinguishing them from true genetic mutations that are permanently inherited.

**139.** (A) I, II and IV
Chemical and physical mutagens are widely used to induce variation in vitro. EMS primarily causes point mutations, while gamma rays result in larger chromosomal changes like deletions. A common challenge in mutagenizing multicellular explants is chimerism, where only some cells carry the mutation. While mutations occur in the M1 generation, recessive traits typically require further generations or haploid systems for detection and stabilization.

**140.** (A) Incorporating the herbicide into the medium at a concentration slightly above the established lethal dose for wild-type cells.
To select for herbicide-resistant cell lines, a selective agent is added directly to the culture medium. The concentration must be high enough to kill all sensitive, wild-type cells. Only those rare cells with a mutation conferring resistance will be able to survive and divide into a callus. This resistant tissue can then be regenerated into a whole plant that inherits the herbicide tolerance trait.

**141.** (A) Plant-pathogen interactions leading to phytoalexin synthesis
The use of biotic elicitors, such as fungal cell wall fragments, mimics a natural pathogen attack on the plant. This triggers the plant's innate defense response, specifically the synthesis of phytoalexins and other protective secondary metabolites. In cell culture, this strategy is employed to artificially stimulate these pathways, leading to much higher yields of valuable compounds than would be produced under normal growth conditions.

**142.** (C) Root inducing
The Ri plasmid, found in the bacterium Agrobacterium rhizogenes, stands for "root-inducing." When this bacterium infects a plant, it transfers a portion of this plasmid into the host's genome. This genetic transformation causes the plant to develop a profusion of highly branched, fast-growing "hairy roots" at the site of infection. These roots are used in biotechnology for the stable production of secondary metabolites.

**143.** (A) P-2, Q-1, R-3, S-4
Different bioreactors use unique mechanisms for mixing cell suspensions. Stirred tank bioreactors use a mechanical impeller for agitation. Bubble columns rely solely on air sparging from the bottom. Airlift bioreactors use gas to drive liquid through a central draft tube for cyclic circulation. Rotating drum bioreactors achieve mixing by the physical rotation of the entire vessel. Each design offers specific advantages for aeration and shear protection.

**144.** (A) Porous support matrix for adsorption/ attachment
Polyurethane foam is a type of porous support used in plant cell immobilization. The open-cell structure of the foam provides a large surface area where plant cells can naturally adhere or become physically trapped. This method of immobilization allows the medium to flow through the foam, facilitating nutrient exchange and product removal while protecting the cells from mechanical stress in a bioreactor environment.

**145.** (A) Both A and R are true and R is the correct explanation of A.
Biotransformation leverages the complex enzymatic machinery of plant cells to perform specific chemical changes on added compounds. Plant enzymes can carry out difficult reactions, such as stereo-specific hydroxylations or glycosylations, which are often challenging to replicate using synthetic chemistry. This allows for the production of high-value pharmaceutical compounds and other specialty chemicals using sustainable, cell-based systems that function as biological catalysts.

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on the plant species and culture conditions, all three pathways are capable of diverting gametophytic development toward the formation of haploid embryos or callus.

**129.** (A) Mature pollen grains (trinucleate) are generally recalcitrant and do not switch from a gametophytic to a sporophytic pathway.
The timing of anther collection is crucial because the microspores must be in a developmentally plastic state to undergo androgenesis. Mature pollen grains are already committed to the gametophytic pathway and have thick walls that make them resistant to reprogramming. In contrast, younger microspores at the uninucleate or early binucleate stage can be more easily induced to become sporophytic and form embryos.

**130.** (A) Parthenogenesis (a form of gynogenesis)
Parthenogenesis is a specific form of gynogenesis where an unfertilized egg cell is induced to develop into an embryo. In the context of tissue culture, this occurs through the in vitro culture of unfertilized ovaries or ovules. This process results in the production of haploid plants from the female gametic line, providing an alternative to androgenesis for creating homozygous doubled haploid lines.

**131.** (A) Transfer of late blight resistance from Solanum demissum to cultivated potato.
Embryo rescue has been successfully used to overcome incompatibility barriers in wide crosses, such as those between cultivated crops and their wild relatives. A classic example is the transfer of late blight resistance from the wild species Solanum demissum into the cultivated potato. By rescuing the hybrid embryos that would otherwise abort, breeders can integrate valuable genetic traits from wild species into modern varieties.

**132.** (B) One male gamete with two polar nuclei.
In most flowering plants, the endosperm is a unique triploid tissue formed during double fertilization. It results from the fusion of one of the two male gametes with the two polar nuclei located in the central cell of the embryo sac. This triploid nature makes endosperm culture an ideal method for regenerating plants with three sets of chromosomes, which often exhibit seedlessness in fruits.

**133.** (A) Both A and R are true and R is the correct explanation of A.
Fluorescein diacetate is a reliable viability stain because its fluorescence depends on metabolic activity and membrane integrity. The non-fluorescent FDA molecule enters the cell and is cleaved by esterases, which are only active in living cells. The resulting fluorescein is trapped inside the intact membrane and glows green under UV light, allowing for the clear differentiation of viable and non-viable protoplasts.

**134.** (A) II, III, I, IV
The process of isolating protoplasts follows a precise order. First, the source tissue is surface sterilized to prevent contamination. Then, the tissue is prepared by peeling the epidermis or slicing to expose the cells. This is followed by incubation in a specialized enzyme mixture to digest the cell walls. Finally, the isolated protoplasts are purified through centrifugation and washed with an osmoticum.

**135.** (B) It forces the protoplasts into alignment, creating 'pearl chains' via dielectrophoresis.
In the process of electrofusion, an alternating current field is applied first. This field induces an electric dipole in the protoplasts, causing them to move and align into "pearl chains" at the points of highest field intensity. This alignment ensures that the protoplasts are in close physical contact, which is essential for the subsequent high-voltage pulse to successfully induce membrane fusion between them.

**136.** (C) Auxotrophic mutant complementation
Auxotrophic complementation is a powerful selection tool for somatic hybrids. In this system, each parental line has a different nutritional requirement and cannot grow on a simple basal medium. However, when the protoplasts fuse, the resulting hybrid cell inherits the functional genes from both parents, allowing it to synthesize all necessary nutrients. Consequently, only the hybrid cells will proliferate on the unsupplemented selection medium.

**137.** (A) A cytoplasmic hybrid containing a hybrid cytoplasm but only one parental nucleus.
A cybrid, or cytoplasmic hybrid, is a cell formed through protoplast fusion that contains the nuclear genome of just one parent and a cytoplasm containing organelles from both parents. This is often achieved by inactivating the nucleus

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plunged into liquid nitrogen to achieve ultra-low temperature storage.

**65.** (A) The developmental stage of the microspores at the time of excision.
The success of anther culture depends heavily on the developmental stage of the microspores. Generally, the late uninucleate to early binucleate stage is the most responsive phase for diverting development from the gametophytic to the sporophytic pathway. If the anthers are collected too early or too late, the frequency of haploid embryo or callus formation is significantly reduced or even abolished.

**66.** (B) Poaceae (Gramineae)
Albinism is a frequent and significant challenge in the anther culture of many species, particularly within the Poaceae family, which includes major cereals like wheat and rice. Regenerated plants from these cultures often lack chlorophyll and cannot survive autotrophically. This phenomenon is thought to be caused by mutations or rearrangements in the plastid genome during the in vitro androgenic development process.

**67.** (C) A is true but R is false.
Ovary culture is indeed a useful alternative for producing haploid plants when anther culture fails or results in albinos. However, the reason provided is incorrect because gynogenesis aims to induce the haploid cells within the embryo sac, such as the egg cell, to divide. It does not involve the somatic layers of the ovary wall undergoing meiosis, as these are already diploid.

**68.** (B) Degeneration of the endosperm leading to embryo starvation
In the hybridization between wheat and rye, the hybrid embryo often begins to develop normally but eventually dies because the endosperm fails to provide the necessary nutrients. This post-zygotic barrier is overcome by embryo rescue, where the immature hybrid embryo is excised from the developing seed and placed on an artificial nutrient medium to ensure its survival and subsequent growth into a seedling.

**69.** (B) Rhozyme HP-150
Protoplast isolation requires a combination of enzymes to break down the different components of the plant cell wall. While cellulases and pectinases are standard, hemicellulases are often needed for more complex walls. Rhozyme HP-150 is a commercial enzyme preparation with high hemicellulase activity, making it particularly effective for degrading the hemicellulose fraction and improving the yield of viable protoplasts in certain species.

**70.** (B) Dead cells absorb the dye because they have lost membrane integrity.
Evans Blue is a non-permeable stain used to determine the viability of plant cells and protoplasts. Living cells with intact plasma membranes exclude the dye and remain unstained. In contrast, dead or damaged cells have compromised membranes that allow the dye to enter the cytoplasm, staining the cell blue. This exclusion principle provides a simple and rapid method for assessing cell health.

**71.** (A) P-1, Q-4, R-2, S-3
DMSO is a common penetrating cryoprotectant used to lower the freezing point inside cells. Glycerol is a viscous penetrating polyol with similar protective properties. Sucrose is a non-penetrating sugar that acts as an external osmoticum to dehydrate cells. Proline is a naturally occurring amino acid that accumulates in plants under stress and provides protection to cellular structures during the cryopreservation process.

**72.** (A) $Ca^{2+}$
During the chemical induction of protoplast fusion, a high pH environment (approximately 10.5) combined with a high concentration of calcium ions is often employed. The calcium ions help to neutralize the negative surface charges on the plasma membranes, allowing the protoplasts to come into close contact. The high pH then alters the membrane properties, facilitating the fusion of the lipid bilayers.

**73.** (D) Fused cytoplasm but distinct, unfused nuclei from both parents.
A heterokaryon is the immediate product of the fusion of two different protoplasts. It is characterized by having a common fused cytoplasm that contains the organelles of both parents, but the two parental nuclei remain distinct and unfused within the cell. For a true somatic hybrid to form, these nuclei must subsequently fuse during the next round of cell division.

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**146.** (B) Encapsulation-dehydration
Encapsulation-dehydration is a cryopreservation technique that combines physical protection with osmotic and air drying. Explants are first embedded in calcium alginate beads and then pre-cultured in a high-sucrose medium. The beads are subsequently subjected to air desiccation to significantly reduce their water content. This dehydration step is critical because it prevents the formation of lethal ice crystals during the subsequent immersion in liquid nitrogen.

**147.** (A) Slow-freezing allows for controlled extracellular ice formation causing protective cellular dehydration, while rapid-freezing prevents ice crystal formation entirely through vitrification.
Slow-freezing and rapid-freezing represent two different physical approaches to cryopreservation. Slow-freezing involves cooling at a controlled rate, which encourages ice to form outside the cells, drawing water out and dehydrating the cytoplasm. Rapid-freezing, often used in vitrification, involves extremely fast cooling that prevents any ice crystals from forming, instead turning the cytoplasm into a stable, non-crystalline glass-like state for safe storage.

**148.** (A) Extend the subculture interval by reducing the cellular metabolic rate.
Minimal-growth storage is a conservation technique used for medium-term maintenance of plant germplasm. By using lower temperatures, reduced light, or osmotic retardants, the rate of plant growth is significantly slowed. This allows the intervals between necessary subculturing to be greatly extended, reducing the labor and costs associated with maintaining large collections of plants in vitro while still keeping them viable.

**149.** (B) It mandates frequent subculturing (every week) to maintain strict genetic stability.
Plant tissue culture repositories aim to minimize the frequency of subculturing, as frequent divisions increase the risk of somaclonal variation and contamination. Instead, they use slow-growth or cryopreservation techniques to keep plants in a state of reduced activity for long periods. These repositories are essential for the safe, ex situ conservation of elite varieties and endangered species, providing a backup to field-based collections.

**150.** (A) Fructose
Fructose is more sensitive to heat than sucrose and can undergo caramelization during autoclaving. When heated excessively, it can break down into various byproducts, some of which may be inhibitory or even toxic to plant tissues. Therefore, when using fructose as a carbon source, care must be taken with the duration of the sterilization cycle, or it should be sterilized by filtration.

**151.** (A) I, II and III
Essential micronutrients are required in trace amounts for vital physiological functions. Iron is indispensable for chlorophyll synthesis and electron transport. Boron is necessary for the integrity of the cell wall and the function of cellular membranes. Molybdenum is a critical cofactor for the enzyme nitrate reductase. Zinc, however, has unique roles in enzyme activation and cannot substitute for the structural functions of calcium.

**152.** (A) EDTA (Ethylenediaminetetraacetic acid)
Iron is prone to forming insoluble precipitates in the pH range typical for plant tissue culture media. To prevent this, it is added in a chelated form, most commonly using EDTA. The EDTA molecules surround the iron ions, keeping them in a soluble state that remains available for uptake by the plant cells even as the pH of the medium fluctuates during the culture period.

**153.** (A) Most bacteria and fungal spores
Membrane filtration using a pore size of 0.22 micrometers is the standard method for sterilizing heat-sensitive liquids in the laboratory. This specific pore size is small enough to physically trap and remove almost all bacteria and fungal spores. However, it is generally not small enough to block viruses, which require much finer filters. This technique ensures that vitamins and growth regulators remain sterile without being degraded.

**154.** (C) 1-5% available chlorine for 10-20 minutes
Sodium hypochlorite is a widely used disinfectant for the surface sterilization of explants. For resilient materials like seeds or woody stems, a concentration containing 1 to 5% available chlorine is typically used. The explants are submerged for 10 to 20 minutes to ensure the destruction of surface-borne microbes. This must be followed by thorough rinsing to prevent the chemical from damaging the plant tissue.

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of sexual reproduction. It allows for the fusion of protoplasts from species that cannot be crossed traditionally due to pre-zygotic or post-zygotic incompatibility. This technique enables the transfer of desirable traits from wild relatives or even unrelated species into cultivated crops, greatly expanding the gene pool available for plant improvement programs.

**172.** (A) Cybridization
Traits that are encoded by the chloroplast genome, such as certain types of herbicide resistance, are inherited maternally through the cytoplasm. Cybridization is the most effective technique for transferring these traits because it involves the fusion of cytoplasm from two parents while keeping the nucleus of only the recipient species. This allows the beneficial chloroplast genes to be integrated into a desirable crop line without altering its nuclear characteristics.

**173.** (A) Molecular marker analysis (e.g., RAPD, AFLP, SSR)
While somaclonal variation can sometimes be seen visually, molecular marker analysis provides a much more precise and objective way to detect genetic changes. Techniques like RAPD, AFLP, and SSR allow researchers to compare the DNA profiles of regenerated plants with the original mother plant. These markers can identify subtle mutations, insertions, or deletions that occurred during the tissue culture process, confirming whether the clones are genetically identical.

**174.** (A) Culturing cells in media supplemented with culture filtrates from a pathogenic fungus.
In vitro selection for biotic stress resistance involves exposing plant cell cultures to selective agents derived from pathogens. Often, the culture filtrate of a pathogenic fungus, which contains toxic metabolites, is added to the growth medium. Only the cells that possess or develop resistance to these toxins will survive and proliferate. These resistant cell lines can then be regenerated into plants with potential field resistance to the pathogen.

**175.** (A) The dose that inhibits 50% of the growth or causes 50% mortality in the treated explant population.
Establishing the LD50 is a fundamental step in mutagenesis because it defines the sensitivity of the tissue to the mutagen. This value indicates the dose of a chemical or physical agent that results in the death or total growth inhibition of half the treated population. Choosing a dose near the LD50 usually provides an optimal balance between a high mutation frequency and the survival of enough cells for regeneration.

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**176.** (D) Immobilization of cells generally decreases the rate of secondary metabolite secretion to zero.
Immobilization of plant cells often enhances rather than decreases the production and secretion of secondary metabolites. By entrapping cells in a matrix like calcium alginate, they are protected from shear stress and maintained in a stable physiological state. This often leads to increased metabolic activity and facilitates the continuous recovery of products secreted into the medium, making it a valuable tool for industrial-scale metabolite production.

**177.** (A) Stage 1 optimizes biomass production; Stage 2 optimizes product synthesis.
A two-stage culture system is a common strategy for optimizing secondary metabolite production. In the first stage, the culture conditions are designed to maximize the rapid accumulation of biomass. Once a sufficient amount of tissue is produced, the cells are transferred to a second stage with a different medium or environment that triggers and maximizes the biosynthesis of the target secondary metabolite, often through the use of elicitors.

**178.** (A) Both A and R are true and R is the correct explanation of A.
Airlift bioreactors are preferred for scaling up plant cell suspensions because they do not have mechanical impellers that cause high shear stress. Plant cells, being relatively large and having rigid cell walls, are highly susceptible to physical damage from the turbulence generated in traditional stirred-tank bioreactors. The gas-driven mixing in airlift systems provides a gentler environment that maintains cell viability while ensuring adequate aeration and nutrient distribution.

**179.** (A) The physical protection against shear stress provided by the matrix.
Immobilized plant cells often exhibit increased longevity and productivity because the surrounding matrix provides a protective barrier. In a bioreactor, cells are subject to hydrodynamic shear forces that can damage the

**163.** (A) Spontaneous doubling occurs at very low and unpredictable frequencies.
While some haploid plants can spontaneously double their chromosome number in tissue culture, this occurs inconsistently and at low rates. In plant breeding, where speed and reliability are essential, chemical doubling using colchicine is preferred. Colchicine ensures that a high percentage of haploid plantlets are successfully converted into fertile, homozygous doubled haploids, allowing for the rapid stabilization of new genetic lines in a single step.

**164.** (A) The frequency of responding ovules is generally much lower than the vast number of responsive microspores per anther.
A major limitation of gynogenesis is its relatively low efficiency compared to androgenesis. Each flower contains only a few ovules, whereas a single anther can produce thousands of microspores. This means that the number of potential haploid plants that can be generated from anther or microspore culture is much higher, making androgenesis the primary choice for haploid production in most crops where it is technically feasible.

**165.** (A) P-2, Q-4, R-1, S-3
Different tissue culture techniques serve specific agricultural goals. Meristem culture is primarily used to produce virus-free plants. Endosperm culture is utilized to generate triploid plants, which are often seedless. Embryo rescue is essential for overcoming post-zygotic barriers in distant hybridization. Anther culture is the most common method for producing homozygous doubled haploids, which significantly accelerates the breeding process for many important crop species.

**166.** (A) Both A and R are true and R is the correct explanation of A.
Wide hybridization between different species often fails because the endosperm does not develop properly to support the hybrid embryo. This failure is usually due to a genomic imbalance that prevents normal double fertilization or subsequent endosperm growth. Without a functional endosperm, the embryo starves and aborts prematurely. Embryo rescue addresses this by providing the necessary nutrients through an artificial medium, allowing the hybrid to survive.

**167.** (A) Callus induction followed by shoot organogenesis
Endosperm is a unique triploid tissue that can be challenging to regenerate in vitro. When successful, the typical pathway involves the initial induction of an unorganized callus from the excised endosperm tissue. Following this, the callus is transferred to a medium with a specific hormonal balance to trigger shoot organogenesis. This process results in the regeneration of triploid plants, which are highly valued for producing seedless fruits.

**168.** (A) The maintenance of an iso-osmotic or slightly hypertonic environment using osmotica.
Maintaining an appropriate osmotic environment is the most critical factor for the survival of naked protoplasts. Without a cell wall, the protoplast is highly vulnerable to osmotic shock. The use of osmotica, such as mannitol or sorbitol, ensures that the external solution has a similar or slightly higher osmotic pressure than the cytoplasm. This prevents the rapid influx of water that would cause the protoplasts to burst.

**169.** (C) Mannitol - Induces spontaneous membrane fusion
In protoplast technology, different chemicals serve specific roles. Cellulase and pectinase are enzymes used for cell wall degradation, while Fluorescein Diacetate is used to assess viability. Mannitol, however, is an osmotic stabilizer used to prevent the lysis of protoplasts. It does not induce membrane fusion. Spontaneous fusion is rare and unpredictable; instead, chemical fusogens like PEG or electrical pulses are used to induce fusion.

**170.** (A) I, II and IV
Electrofusion is a sophisticated technique for merging protoplasts. It uses dielectrophoresis to align the cells into chains, followed by high-voltage DC pulses to induce reversible membrane breakdown and subsequent fusion. This method is often less toxic than chemical fusion and allows for the precise, targeted fusion of specific pairs of cells under a microscope. It is a highly efficient tool for creating unique somatic hybrids and cybrids.

**171.** (A) Allows for the combination of genomes from sexually incompatible species.
The most significant advantage of somatic hybridization is its ability to bypass the barriers

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which involve modifications to gene expression without altering the underlying DNA sequence.

**206.** (C) A is true but R is false.
Fusaric acid is a toxin produced by the fungus Fusarium and can be used as a selective agent to develop disease-resistant plants. However, the reason provided is incorrect. Fusaric acid is not a mutagen; instead, it acts as a selective pressure. Only the cells that already possess a natural mutation or resistance mechanism will survive the toxin, allowing researchers to isolate and regenerate these resistant lines for breeding.

**207.** (A) Gamma rays from a Cobalt-60 source
Physical mutagenesis involves the use of ionizing radiation to induce genetic changes in plant tissues. Gamma rays, typically emitted from a Cobalt-60 source, are one of the most common physical mutagens used in tissue culture. These high-energy rays penetrate deep into the tissues and cause double-strand breaks in the DNA, leading to various types of mutations and chromosomal rearrangements that can be screened for desirable traits in plant breeding.

**208.** (A) Plagiotropic growth habit and lack of geotropism.
Hairy roots, induced by Agrobacterium rhizogenes, have a distinct growth pattern that sets them apart from normal roots. They exhibit plagiotropic growth, meaning they grow horizontally rather than following the downward pull of gravity. Additionally, they are characterized by high levels of branching and rapid growth on media lacking growth regulators. These unique traits make hairy roots highly efficient systems for the stable production of plant secondary metabolites.

**209.** (A) I, II and IV
Elicitors are used in plant biotechnology to stimulate the production of secondary metabolites. Biotic elicitors like fungal cell wall fragments mimic a pathogen attack, while abiotic elicitors like UV light and heavy metals create environmental stress. Both types trigger the plant's defense pathways, leading to the accumulation of protective compounds. Elicitation is typically performed during the late log or early stationary phase to maximize product yield after biomass accumulation.

**210.** (A) Airlift bioreactor
The airlift bioreactor is specifically designed for the cultivation of shear-sensitive cells. It uses a gas stream to drive the circulation of the medium through a central riser tube and a downcomer zone. This configuration ensures that nutrients and oxygen are distributed evenly throughout the vessel while the cells are protected from the mechanical damage that can occur in stirred-tank systems. It is highly effective for scaling up plant cell suspensions.

**211.** (A) To facilitate continuous operation and easy separation of cells from the product-containing medium.
Plant cell immobilization is a valuable technique for industrial production. By entrapping cells in a matrix, they can be maintained in a stable environment and reused for multiple production cycles. This approach allows for the continuous flow of medium, where the secreted products can be harvested without destroying the cell biomass. Immobilization also provides protection against mechanical stress and simplifies the downstream processing of the target metabolites.

**212.** (A) DMSO enters the cell to reduce the freezing point and prevent internal ice, whereas sucrose acts externally to osmotically dehydrate the cell.
Cryoprotectants are classified into penetrating and non-penetrating types. DMSO is a penetrating agent that moves into the cell to lower the freezing point of the cytoplasm. Sucrose is a non-penetrating agent that stays outside the cell, creating an osmotic gradient that draws water out, thereby dehydrating the cell before freezing. Using a combination of both types provides the best protection against the damaging effects of ice crystal formation during cryopreservation.

**213.** (A) I, III, IV, II
A standard vitrification protocol begins with the excision of the shoot tip, followed by a pre-culture period on a medium enriched with sucrose to enhance osmotic tolerance. The tissue is then exposed to a highly concentrated Plant Vitrification Solution, such as PVS2, to severely dehydrate the cells and increase their viscosity. Finally, the treated shoot tips are rapidly plunged into liquid nitrogen to achieve a stable glassy state without ice formation.

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plasma membrane. The immobilization matrix, such as alginate, cushions the cells against these forces. This stable environment allows the cells to maintain their metabolic activity for much longer periods than they would in free suspension.

**180.** (A) To severely dehydrate the cells and increase cytoplasmic viscosity, ensuring a glass transition upon freezing.
In vitrification protocols, the Plant Vitrification Solution is a highly concentrated mixture of cryoprotectants. Its role is to osmotically remove most of the water from the cells, which significantly increases the viscosity of the remaining cytoplasm. When the tissue is rapidly cooled, this viscous state prevents ice from crystallizing and instead leads to a stable, glassy transition, protecting the cellular integrity during storage in liquid nitrogen.

**181.** (A) Dimethyl sulfoxide (DMSO)
Dimethyl sulfoxide is the most widely used cryoprotectant in plant biotechnology. It is a penetrating agent that moves across the plasma membrane into the cytoplasm. DMSO works by forming hydrogen bonds with water molecules, which lowers the freezing point and interferes with the formation of large, destructive ice crystals. This chemical is essential for ensuring high survival rates during the slow-freezing of berbagai plant tissues and cell lines.

**182.** (A) I, II and IV
Slow-growth germplasm conservation is a practical method for maintaining collections of clonal crops like potato and banana. It relies on reducing the temperature and using osmotic retardants to slow down the rate of growth. While this method significantly extends the time between subcultures, it is not an indefinite solution like cryopreservation, as the cells remain metabolically active and must still be subcultured periodically to maintain health.

**183.** (A) Ammonium nitrate
Ammonium nitrate is the primary source of reduced nitrogen in the MS medium. Nitrogen is an essential element for the synthesis of proteins, amino acids, and nucleic acids. The combination of ammonium and nitrate ions in the medium provides a versatile nitrogen source that plant cells can readily utilize. This specific nitrogen balance is one of the key factors that makes MS medium so effective for a wide range of species.

**184.** (C) Vitamins function primarily as co-factors for critical enzymatic reactions.
Vitamins are essential organic compounds required in small amounts as co-factors for various enzymatic reactions in plant metabolism. While thiamine is an absolute requirement for most cultures, other vitamins like niacin or pyridoxine often enhance growth without being strictly essential for all species. Myo-inositol, although often grouped with vitamins, is a sugar alcohol that plays a fundamental structural role in cell wall and membrane biosynthesis.

**185.** (A) $ZnSO_4$
Zinc sulfate is a micronutrient in plant tissue culture media, meaning it is required in very small, trace amounts. Other micronutrients include boric acid, manganese sulfate, and copper sulfate. In contrast, compounds like potassium phosphate, magnesium sulfate, and calcium chloride are macronutrients, which are needed in much larger quantities to provide the essential building blocks for cellular structure and function in the growing plant tissue.

**186.** (A) II, I, III, IV
The preparation of heat-labile growth regulators follows a specific sequence. First, the PGR solution is sterilized using a fine membrane filter. Meanwhile, the basal agar medium is autoclaved and allowed to cool to a temperature that will not degrade the PGR. Then, the filtered solution is added aseptically to the warm, liquid agar. Finally, the mixture is gently combined and poured into sterile culture vessels for use.

**187.** (A) Coagulate and precipitate cellular proteins by interacting with sulfhydryl groups.
Mercuric chloride is an extremely effective but toxic surface sterilant. Its microbicidal action is based on the ability of mercury ions to bind strongly to sulfhydryl groups in cellular proteins. This interaction leads to the coagulation and precipitation of proteins, which effectively inactivates the enzymes and metabolic pathways of microorganisms. Because of its high toxicity, it must be completely rinsed away from the explant after treatment.

**188.** (A) Both A and R are true and R is the correct explanation of A.
For a mature plant cell to give rise to a new structure in tissue culture, it must first undergo dedifferentiation. This process involves the cell

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**223.** (A) I, II and III
Single-cell culture techniques allow researchers to study cell development and genetic variation. Cells can be isolated mechanically or through enzymatic digestion of the cell wall. Methods like the micro-chamber technique use nurse tissues to provide essential growth factors for isolated cells. Bergmann's plating involves embedding cells in agar for spatial separation. However, single cells generally require small, concentrated volumes of media to maintain a high enough density of secreted factors for division.

**224.** (A) The emergence of bilateral symmetry due to the initiation of cotyledon primordia.
The transition from the globular stage to the heart stage is a fundamental morphological shift during somatic embryogenesis. It is marked by the change from a radially symmetrical sphere of cells to a bilaterally symmetrical structure. This change occurs as the cotyledon primordia begin to develop at the apical end of the embryo, giving it a heart-like shape and establishing the future body plan of the plant.

**225.** (A) The intervening callus phase significantly increases the risk of somaclonal variation.
Indirect somatic embryogenesis involves a period of undifferentiated growth as a callus before embryos are formed. This prolonged period of rapid cell division is a major source of genetic and epigenetic instability. As a result, plants regenerated via this pathway are much more likely to exhibit somaclonal variation. For clonal propagation, where uniformity is essential, direct embryogenesis is preferred because it minimizes the risk of these unpredictable changes.

**226.** (B) Diploid, $2n = 2x = 24$ (Homozygous)
Anther culture produces haploid plants with half the chromosome number of the parent. In this case, the haploid would have 12 chromosomes. Treating this haploid with colchicine doubles the chromosome set, resulting in a diploid plant with 24 chromosomes. Because the second set of chromosomes is a perfect copy of the first, the resulting plant is completely homozygous at every genetic locus, which is highly valuable for breeding programs.

**227.** (A) Both A and R are true and R is the correct explanation of A.
Microspore culture is often more efficient than anther culture because it uses isolated pollen grains. In anther culture, there is a risk that the surrounding diploid somatic tissues of the anther wall will also divide and regenerate into plants. These plants would be heterozygous diploids, not the desired homozygous doubled haploids. Microspore culture avoids this by starting only with the haploid gametic cells, ensuring that all regenerated plants are of gametic origin.

**228.** (A) II, III, IV, I
The production of doubled haploids follows a logical order. It begins with the cold pretreatment of floral buds to stress the microspores. Then, the anthers are excised and cultured on an induction medium to trigger embryogenesis. Once haploid plantlets are regenerated, they are treated with colchicine to double their chromosome number. This process results in a fertile, homozygous diploid plant that can be used immediately in breeding or genetic research.

**229.** (A) It is the universally preferred primary method for haploid production in all Poaceae crops.
Although gynogenesis is a valuable technique, it is not the primary method for most cereal crops. Anther and microspore culture are generally preferred because each flower produces thousands of microspores, offering a much higher frequency of success. Gynogenesis is typically used as a backup method when androgenesis results in a high frequency of albinism or when the species is recalcitrant to anther culture techniques.

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**230.** (A) Strictly requires a highly complex medium often supplemented with specific amino acids.
The younger an embryo is at the time of excision, the more complex its nutritional requirements. Embryos at the globular stage are particularly delicate and have not yet developed the ability to synthesize many essential compounds. Therefore, they require a highly enriched medium containing vitamins, amino acids, and other organic supplements to survive. As the embryo matures, its nutritional needs become simpler, and it can eventually grow on a basic basal medium.

**231.** (A) A combination of auxin and cytokinin
Triploid production from endosperm culture typically requires the presence of both auxins and cytokinins in the medium. These growth regulators are necessary to induce the differentiated endosperm tissue to first

diverted from its normal gametophytic pathway toward a sporophytic pathway that leads to embryo formation. This stage is universally recognized as the most responsive phase for producing hapioid plants in cereal crops like wheat, rice, and barley.

**198.** (A) Both A and R are true and R is the correct explanation of A.
Cold pretreatment is a common and effective stress treatment used to enhance the success of anther culture. By exposing the floral buds to low temperatures, the normal maturation of the microspores is delayed. This stress triggers a switch in the developmental program, promoting the symmetrical division of the microspore instead of the normal asymmetrical division. This symmetrical division is the first step toward the formation of a haploid embryo.

**199.** (A) I, II and IV
Embryo rescue is a powerful tool for recovering hybrids from incompatible crosses. Immature embryos are more physiologically demanding than mature ones and require a more complex medium, often including organic supplements. The procedure must be done aseptically to prevent contamination. While embryo rescue ensures the survival of the hybrid embryo, it does not guarantee that the resulting plant will be fertile, as chromosomal imbalances may still cause sterility.

**200.** (A) Production of completely seedless fruits due to highly irregular meiosis.
The primary advantage of triploid plants in agriculture is their sterility, which results from the presence of an odd number of chromosome sets. During meiosis, the three sets of chromosomes cannot pair correctly, leading to non-viable gametes. In many fruit crops, this sterility prevents the formation of seeds. Consequently, triploid varieties are the basis for producing seedless watermelons, citrus, and other popular commercial fruit varieties.

**201.** (A) P-3, Q-1, R-2, S-4
Protoplast fusion can be achieved through various chemical and physical methods. High pH and calcium ions neutralize surface charges, while PEG acts as a molecular bridge to induce membrane agglutination. Electrofusion uses dielectrophoresis for cell alignment and high-voltage pulses for membrane breakdown. Dextran sulfate is another chemical fusogen that uses electrostatic interactions. Each method aims to bring membranes into close enough contact for the lipid bilayers to merge.

**202.** (A) To cause slight plasmolysis, shrinking the protoplast away from the cell wall and preventing osmotic lysis.
Adding an osmoticum to the enzyme solution serves a dual purpose during protoplast isolation. First, it causes the cell to undergo slight plasmolysis, which helps to separate the plasma membrane from the cell wall, making it easier for the enzymes to work. Second, it maintains an osmotic balance that prevents the fragile protoplast from taking up too much water and bursting once the rigid cell wall is removed.

**203.** (A) It is extensively used to transfer partial organelle genomes without touching the nucleus.
Symmetric somatic hybridization involves the complete fusion of two intact nuclei, resulting in a hybrid that contains the full nuclear genome of both parents. This technique is used to combine nuclear traits from different species. In contrast, transferring organelle genomes without the full nucleus is the goal of cybridization or asymmetric hybridization. Symmetric hybrids typically possess the sum of the parental chromosome numbers and are used to create wide-hybrid plants.

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**204.** (A) Double mutant complementation
Double mutant complementation is a reliable method for selecting somatic hybrids after fusion. If Parent A is resistant to antibiotic X but sensitive to Y, and Parent B is resistant to Y but sensitive to X, neither parent can grow on a medium containing both X and Y. However, a hybrid cell that inherits both resistance genes will survive and grow, allowing for the easy and efficient selection of true hybrid plants.

**205.** (A) Chromosomal translocations and aneuploidy
Genetic somaclonal variation involves permanent changes to the DNA or chromosome structure. Common examples include chromosomal translocations, where pieces of chromosomes are moved, and aneuploidy, which is the gain or loss of individual chromosomes. These genetic changes are typically irreversible and are inherited by the progeny of the regenerated plants. This differs from epigenetic changes,

losing its specialized characteristics and reverting to a meristematic state. Once dedifferentiated, the cell regains the ability to divide and form an unorganized mass of cells known as a callus. This stage is a critical first step for redirecting the cell toward new developmental pathways.

**189.** (A) Unorganized callus proliferation
The Skoog-Miller model predicts that the relative ratio of auxin to cytokinin determines the direction of differentiation. When the concentrations of both growth regulators are balanced and present at moderate levels, the cells are typically maintained in an undifferentiated state. This leads to the proliferation of a callus, which is a mass of unorganized, actively dividing cells, rather than the induction of specific organs like shoots or roots.

**190.** (A) Somatic embryos
Protocorm-like bodies are structures commonly formed during the micropropagation of orchids. They are functionally analogous to somatic embryos because they are bipolar structures that can develop into complete plantlets. Like somatic embryos, PLBs can be induced directly from explants or indirectly from callus. This pathway is the primary method for the large-scale clonal multiplication of many commercially important orchid species and varieties.

**191.** (A) Rooting of the in vitro generated shoots
Stage III of micropropagation is specifically focused on the induction and development of roots on the shoots produced during the multiplication phase. Shoots are typically transferred to a medium containing a higher concentration of auxin, which signals the formation of a root system. Successful rooting is essential for the plantlets to become self-sufficient and survive the subsequent transfer to soil during the acclimatization phase.

**192.** (B) Cultivation in a growth chamber at $35 - 40^{\circ}C$ for 3 to 4 weeks
Thermotherapy is a standard procedure for eliminating viruses from infected plant materials. By maintaining the mother plants or in vitro cultures at elevated temperatures, typically between 35 and 40 degrees Celsius, for several weeks, the replication of many viruses is severely restricted. This creates a zone of virus-free tissue at the shoot tip, which can then be excised and cultured to regenerate completely healthy plants.

**193.** (C) Stationary phase - Exponential increase in primary metabolites
Statement C is incorrect because the stationary phase is characterized by a cessation of growth rather than an exponential increase. During this phase, cell division and biomass accumulation stop due to nutrient depletion or the buildup of inhibitors. While secondary metabolite production often peaks during the stationary phase, primary metabolic activity decreases as the culture reaches the end of its active growth cycle and prepares for senescence.

**194.** (A) Exhibit bipolarity, possessing both an apical and a basal meristem simultaneously.
Somatic embryos are unique because they are bipolar structures, meaning they possess both a shoot apical meristem and a root apical meristem from an early stage of development. This distinguishes them from organogenesis, where either a shoot or a root is formed independently. Somatic embryos develop as independent entities with no direct vascular connection to the maternal tissue, making them structurally and functionally similar to zygotic embryos.

**195.** (A) 2,4-D (2,4-Dichlorophenoxyacetic acid)
2,4-D is a powerful synthetic auxin that is widely considered the most effective hormone for the induction of somatic embryogenesis. It is used to trigger the formation of pro-embryogenic masses in a vast range of plant species. Once these masses are formed, the 2,4-D is usually removed or its concentration is significantly reduced to allow the embryos to complete their development and mature into plantlets.

**196.** (A) Induce a state of quiescence similar to natural orthodox seeds.
Desiccation is a critical step in the production of certain types of artificial seeds. By slowly removing water from the encapsulated somatic embryos, they are induced to enter a state of metabolic quiescence. This process mimics the natural maturation of orthodox seeds, which become dry and dormant. Quiescent synthetic seeds are much easier to store and transport for extended periods compared to hydrated beads.

**197.** (A) Late uninucleate to early binucleate stage
The late uninucleate to early binucleate stage is the most critical window for the induction of androgenesis. At this developmental point, the microspore is still plastic and can be easily

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**214.** (A) It requires extensive infrastructure similar to liquid nitrogen cryogenics.
Slow-growth germplasm storage is a relatively simple and cost-effective method of conservation. Unlike cryopreservation, it does not require liquid nitrogen or specialized cryogenic equipment. Instead, it uses standard culture facilities where the temperature and light are reduced to slow down the growth of the plants. This makes it an ideal method for the medium-term maintenance of clonal collections, although it still requires periodic subculturing to ensure health.

**215.** (A) Both A and R are true and R is the correct explanation of A.
Managing a germplasm repository requires ensuring that the stored materials are completely free of pathogens. It is critical to index for latent viruses because cryopreservation and slow-growth storage do not eliminate existing viral infections. If infected tissue is stored, the regenerated plants will still carry the virus. Therefore, only certified virus-free materials should be entered into long-term storage to prevent the spread of diseases during future propagation.

**216.** (A) $H_3BO_3$ (Boric acid)
Boric acid is the specific compound used to provide boron in the Murashige and Skoog medium and many other formulations. Boron is an essential micronutrient required for cell wall synthesis, membrane function, and the transport of sugars within the plant. Because it is needed in very small amounts, boric acid is typically included in a concentrated micronutrient stock solution during the preparation of the culture medium.

**217.** (A) Activated charcoal or ascorbic acid
Tissue browning is a common problem caused by the leakage and subsequent oxidation of phenolic compounds from damaged explants. To prevent this, antioxidants like ascorbic acid are added to the medium to inhibit oxidation. Alternatively, activated charcoal can be used to adsorb the phenolics and their toxic byproducts. These additives help to create a healthier environment for the explant to establish and grow without suffering from necrosis.

**218.** (A) Antibiotics like kanamycin and hygromycin
Antibiotics are essential for selection in genetic transformation, but they are generally heat-sensitive and would be destroyed by autoclaving. Therefore, solutions of kanamycin, hygromycin, and other antibiotics must be sterilized using a fine membrane filter. These sterile solutions are then added to the autoclaved and cooled medium just before it is poured into plates, ensuring that the antibiotics remain active for the selection process.

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**219.** (A) Mercuric chloride requires zero rinsing because it is highly volatile and non-toxic.
Mercuric chloride is an extremely toxic chemical that is not volatile. It must be handled with great care and requires multiple, thorough rinses with sterile distilled water to ensure that every trace of the chemical is removed from the explant. Any residual mercury is highly phytotoxic and will lead to the death of the plant tissue. This toxicity also poses a significant risk to the researcher and the environment.

**220.** (A) P-3, Q-1, R-4, S-2
Micropropagation is divided into several stages. Stage I involves the initiation of an aseptic culture from the explant. Stage II is dedicated to the rapid multiplication of shoots. Stage III is the rooting phase, where shoots are moved to a different medium to induce roots. Finally, Stage IV involves the transfer of the in vitro plantlets to the greenhouse for hardening and acclimatization to natural environmental conditions.

**221.** (A) Meristem tip culture
Meristem tip culture is uniquely effective for producing virus-free plants because the vascular system does not extend into the very tip of the shoot apical meristem. Viruses typically move through the plant via the phloem, and since the meristem lacks these connections, it often remains uninfected. By carefully excising and culturing just the meristematic dome, researchers can regenerate a healthy plant that is completely free of systemic viruses.

**222.** (C) Continuous agitation on an orbital shaker
To establish a cell suspension culture, a friable callus is placed into a liquid medium and kept under continuous agitation on an orbital shaker. The physical movement of the shaker provides aeration and creates gentle mechanical forces that help to break the callus into smaller clusters and single cells. This constant agitation is essential for maintaining the cells in suspension and ensuring they have access to nutrients and oxygen.

**155.** (A) Both A and R are true and R is the correct explanation of A.
Cellular totipotency is the biological principle that allows a whole plant to be regenerated from a single cell. The Skoog-Miller model provides the practical framework for this by showing that the developmental fate of cells can be controlled using specific ratios of auxin and cytokinin. This allows researchers to direct undifferentiated callus cells to form either shoots or roots, demonstrating the practical application of totipotency.

**156.** (A) Tissues appearing water-soaked, translucent, and possessing poor stomatal function.
Hyperhydricity, previously known as vitrification, is a physiological disorder that occurs in in vitro plantlets. It is characterized by an excessive accumulation of water in the tissues, giving them a swollen, translucent, and brittle appearance. These plants often have poorly developed cuticles and non-functional stomata, which makes them very difficult to acclimatize to ex vitro conditions, often resulting in high mortality during transfer.

**157.** (A) Developing a functional waxy cuticle and capable stomatal regulatory mechanisms.
During the acclimatization phase, plantlets must adapt to lower humidity and higher light intensity. The most critical changes are the development of a thick waxy cuticle to prevent water loss and the establishment of functional stomata that can open and close correctly. These physiological adaptations are essential for the plant to maintain its water balance and survive in the natural, non-sterile environment outside the culture vessel.

**158.** (B) The initial period of adaptation where cell mass increases without significant cell division.
The lag phase is the first stage of growth after cells are transferred to a fresh medium. During this period, the cells are not yet dividing but are metabolically active, accumulating ATP and synthesizing the enzymes and proteins needed for growth. It is a period of physiological adjustment to the new environment. The duration of this phase depends on the state of the inoculum and the medium composition.

**159.** (A) To allow single cells to remain suspended yet spatially separated while still permitting nutrient diffusion.
In Bergmann's plating technique, a low concentration of agar is used to create a semi-solid environment. This concentration is high enough to keep individual cells from sinking or clumping together, ensuring they remain spatially separated in the Petri dish. At the same time, it is low enough to allow for the efficient diffusion of nutrients and growth regulators to each cell, facilitating the formation of isolated colonies.

**160.** (A) II, III, I, IV
Indirect somatic embryogenesis follows a multi-step sequence. First, a suitable explant is selected and placed on a medium with high levels of auxin, such as 2,4-D, to induce an embryogenic callus. Once the callus is established, it is transferred to a low-auxin or auxin-free medium to allow the embryos to develop. Finally, the matured embryos are moved to a germination medium to grow into plantlets.

**161.** (A) Calcium chrome
The production of artificial seeds involves an ion-exchange reaction between sodium alginate and calcium chloride. When a drop of sodium alginate solution containing a somatic embryo enters a calcium chloride bath, the sodium ions are replaced by calcium ions. This cross-links the alginate chains, forming a solid, protective gel bead around the embryo. This encapsulation provides mechanical protection and serves as a delivery system for nutrients.

**162.** (B) The encapsulation matrix cannot support the addition of any beneficial microbes or mycorrhizae.
Artificial seeds are versatile because the encapsulation matrix can be customized with various additives. Nutrients, fungicides, and even beneficial microorganisms like mycorrhizae or growth-promoting bacteria can be incorporated into the alginate gel. This provides the enclosed embryo with a tailored microenvironment that supports its survival and growth upon sowing, making them a useful tool for propagating elite and sensitive plant materials clonally.

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form a callus and then undergo organogenesis or embryogenesis. The specific balance and concentration of these hormones are critical for successfully regenerating triploid plants, which are often used to produce seedless varieties in horticultural crops like grapes and citrus.

**232.** (A) P-1, Q-2, R-3, S-4
Commercial enzymes used for protoplast isolation are derived from various fungal sources. Cellulase Onozuka is sourced from the fungus Trichoderma viride. Macerozyme and Pectolyase are obtained from Rhizopus and Aspergillus species, respectively, and are used to digest the pectin in the cell wall. Driselase is a complex enzyme mixture from basidiomycete fungi. These preparations are essential for efficiently removing the rigid plant cell wall to produce viable protoplasts.

**233.** (A) Fluorescein leaks out of the cell because the plasma membrane loses integrity, and the dye is subject to photobreaching.
The fading of fluorescence during FDA staining is a common phenomenon. Prolonged exposure to high-intensity UV light can cause photobreaching, which chemically degrades the fluorescein molecules. Additionally, the energy from the UV light can damage the plasma membrane of the protoplast. Once the membrane's integrity is compromised, the trapped fluorescein leaks out into the surrounding medium, causing the cell to lose its characteristic green glow under the microscope.

**234.** (A) I, II and IV
Cybridization is used to transfer organelle-encoded traits by creating a cell with a hybrid cytoplasm. This process requires the inactivation of the nucleus of one parent, often achieved through gamma or X-ray irradiation. The resulting cybrid contains the nuclear genome of only the recipient parent, ensuring that nuclear traits are not mixed. This is a powerful tool for integrating mitochondrial or chloroplast-borne characteristics like cytoplasmic male sterility into elite crop lines.

**235.** (A) The fusion of two protoplasts originating from the same parental species or line.
A homokaryon is a fused cell that contains two or more nuclei that are genetically identical because they came from the same parental source. This occurs during protoplast fusion when two cells of the same species or variety merge. While homokaryons are interesting for studying membrane fusion, they do not provide the genetic diversity that breeders seek. Instead, the goal is usually to create heterokaryons, which contain nuclei from different parents.

**236.** (A) Both A and R are true and R is the correct explanation of A.
Transposable elements, or "jumping genes," are normally silenced in the plant genome. However, the severe physiological stress of tissue culture can cause these elements to become active. They may excise themselves and reinsert into new locations, potentially disrupting gene sequences or altering expression levels. This activation is a significant source of genetic somaclonal variation, leading to the unpredictable phenotypic changes often observed in plants regenerated from in vitro cultures.

**237.** (A) In vitro selection
In vitro selection is a technique used to identify cell lines with specific tolerances. By adding a selective agent, such as a heavy metal, to the culture medium, only the cells that have the physiological or genetic capacity to tolerate that stress will survive. These surviving cells are then subcultured and regenerated into plants. This approach allows researchers to rapidly screen millions of cells for rare mutations conferring tolerance to abiotic or biotic stresses.

NODIA

**238.** (A) Doses significantly above LD50 cause excessive tissue necrosis and prevent plant regeneration.
Establishing the LD50 is critical because it ensures that the mutagen dose is high enough to be effective but not so high that it destroys the tissue's regenerative capacity. If the dose is too extreme, the cells suffer from massive DNA damage and physiological collapse, leading to widespread necrosis. A dose near the LD50 provides a high probability of inducing useful mutations while still allowing enough surviving cells to regenerate into whole plants.

**239.** (A) Phytoalexins
Phytoalexins are antimicrobial compounds synthesized by plants in response to stress or attack by pathogens. In tissue culture, the production of these secondary metabolites can be artificially induced by adding elicitors to the medium. Elicitors trigger the plant's defense

2009

**61.** How do α-amylase inhibitors confer insect resistance?
(A) By binding to the midgut epithelium and forming pores
(B) By inhibiting the insect molting hormone
(C) By destroying the insect's nervous system
(D) By blocking the insect's ability to digest starch

2017

**62.** Which regulatory protein is a central hub for the systemic acquired resistance (SAR) pathway and is often manipulated for broad-spectrum disease resistance?
(A) P5CS
(B) DREB1
(C) NPR1
(D) ACC synthase

2021

**63.** Choline monooxygenase is an enzyme involved in the biosynthesis of which specific osmoprotectant?
(A) Proline
(B) Mannitol
(C) Trehalose
(D) Glycine βine

2001

**64.** The bxn gene is introduced into crops to provide resistance against which specific herbicide?
(A) Bromoxynil
(B) Glyphosate
(C) Glufosinate
(D) Atrazine

2015

**65.** The mutation in the EPSPS gene conferring glyphosate tolerance prevents the herbicide from:
(A) Degrading the EPSPS enzyme
(B) Binding competitively to the EPSPS active site
(C) Methylating the EPSPS promoter
(D) Transporting the enzyme into the chloroplast

2024

**66.** Overexpression of ACC deaminase in plants delays fruit ripening by:
(A) Enhancing the production of ethylene
(B) Blocking the receptors that sense ethylene
(C) Degrading ACC, the precursor of ethylene
(D) Modifying the cell wall structure directly

2006

**67.** The primary goal of expressing a ferritin gene in transgenic plants is to:
(A) Increase starch accumulation in the sink organs
(B) Detoxify heavy metals in contaminated soils
(C) Enhance resistance against bacterial blight
(D) Sequester and increase internal iron content

2006

**68.** The modification of plant lipid profiles by silencing the FAD2 gene results primarily in the accumulation of:
(A) Oleic acid
(B) Linoleic acid
(C) Linolenic acid
(D) Palmitic acid

2024

**69.** Which feature of the chloroplast genome allows for the simultaneous expression of multiple transgenes from a single promoter?
(A) Random integration
(B) Maternal inheritance
(C) Polycistronic transcription
(D) RNA interference

2016

**70.** Match the elements of a gene construct in List I with their corresponding function in List II.
List I: (Gene Element)
P. Rubisco small subunit promoter
Q. nptII gene
R. gusA gene
S. MARs
List II: (Function)
1. Minimizes transgene silencing
2. Visual confirmation of expression
3. Directs green tissue-specific expression
4. Confers kanamycin resistance
(A) P-III, Q-IV, R-II, S-I
(B) P-IV, Q-III, R-I, S-II
(C) P-III, Q-I, R-IV, S-II
(D) P-I, Q-IV, R-II, S-III

2006

**71.** In the context of transgene silencing, Post-Transcriptional Gene Silencing (PTGS) involves:
(A) Prevention of mRNA synthesis by RNA polymerase
(B) Sequence-specific degradation of homologous mRNA
(C) Mutation of the transgene sequence
(D) Chromatin remodeling and condensation

2008

**72.** Co-transformation-mediated segregation is a technique utilized to:
(A) Increase the copy number of a transgene
(B) Achieve tissue-specific expression of two

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**247.** (A) Anthers and microspores, often containing a specific balance of iron and varying sucrose levels.
Nitsch's medium was developed specifically to optimize the androgenic response in anther and microspore culture. It features a unique formulation of mineral salts and vitamins that supports the induction of embryos from haploid gametic cells. This medium often includes adjusted levels of iron and sucrose to meet the specific physiological needs of the microspores as they transition from a gametophytic to a sporophytic developmental pathway, particularly in species like tobacco.

**248.** (A) Hyperhydricity and the formation of clustered, stunted, or distorted shoots.
Cytokinins are essential for shoot multiplication, but their excessive use can lead to serious physiological problems. High concentrations over multiple subcultures can disrupt normal growth, leading to hyperhydricity, where the tissues become water-soaked and brittle. This also frequently results in the development of many small, malformed, or clustered shoots that fail to elongate properly. Managing hormone levels is therefore critical for maintaining the quality and health of micropropagated plants.

NODIA

**249.** (A) The hardening of the bead occurs instantaneously upon contact with a calcium chloride solution via ion exchange.
The production of artificial seeds relies on the rapid cross-linking of sodium alginate when it comes into contact with calcium ions. This ion-exchange reaction happens almost immediately as the alginate droplet enters the calcium chloride bath. The calcium ions replace the sodium ions, creating a solid, protective hydrogel bead. This process is gentle and does not damage the sensitive embryo inside, providing a robust and practical method for encapsulating plant propagules for propagation.

**250.** (A) Symmetrical division of the microspore followed by spontaneous chromosome endoreduplication during early embryogenesis.
In some cases, anther culture can yield homozygous diploid plants without the addition of colchicine. This occurs through spontaneous doubling, where the chromosomes replicate without cell division in the early stages of embryo development. This process of endoreduplication naturally doubles the haploid genome, creating a fertile diploid. Because both sets of chromosomes come from the same haploid cell, the resulting plant is completely homozygous, which is highly beneficial for plant breeders.

**********

pathways, leading to the rapid accumulation of phytoalexins. This strategy is widely used in biotechnology to enhance the yield of valuable medicinal and industrial compounds from plant cell suspensions.

**240.** (A) Altered cellular sensitivity and synthesis of endogenous auxins governed by the transferred 'rol' genes.
Hairy root cultures grow rapidly on hormone-free media because they have been genetically transformed by Agrobacterium rhizogenes. The transferred 'rol' genes increase the sensitivity of the plant cells to their own endogenous auxins. This heightened responsiveness triggers continuous, unorganized root growth and branching. The result is a stable, fast-growing biomass that can be maintained indefinitely and is ideal for the large-scale production of specific secondary metabolites and recombinant proteins.

**241.** (A) II, IV, I, III
Scaling up a plant cell culture follows a specific progression. It begins with establishing a friable callus from a chosen explant. Small clusters of these cells are then moved to shake flasks to initiate a liquid suspension. Once the suspension is established and optimized at a small scale, it is used as an inoculum for a larger bioreactor. Finally, the cells or their secreted products are harvested after the culture reaches the desired density.

**242.** (A) Enzymatic conversion of an exogenously supplied precursor into a high-value specific chemical product by cultured cells.
Biotransformation is a biological process where cultured cells act as catalysts to transform one chemical into another. By adding a specific precursor to the medium, the researcher takes advantage of the plant's existing enzymatic pathways to perform complex structural modifications. This technique is especially useful for producing specialized pharmaceutical compounds and flavors that are difficult to synthesize chemically, leveraging the stereo-specificity and efficiency of natural plant enzymes for high-value production.

**243.** (A) II, III and IV
Cryoprotectants are essential for preventing freezing injury. Glycerol is a penetrating agent that helps to stabilize membranes and lower the freezing point. These compounds work by increasing the viscosity of the cytoplasm and interfering with ice crystal formation. Proline is a naturally occurring amino acid that provides additional osmoprotection. While many cryoprotectants are useful at low temperatures, some can be toxic at room temperature, so they must be applied with care.

**244.** (A) To allow time for water to exit the cell as extracellular ice forms, safely dehydrating the cytoplasm.
Slow-freezing is a controlled dehydration process. By cooling the samples at a slow, steady rate, ice crystals are encouraged to form in the surrounding medium first. This increases the osmotic pressure outside the cells, which draws water out of the cytoplasm. This protective dehydration prevents large, lethal ice crystals from forming inside the cell. By the time the samples are plunged into liquid nitrogen, the cells are sufficiently dehydrated to survive the ultra-low temperature.

**245.** (A) P-4, Q-2, R-1, S-3
Germplasm conservation methods are chosen based on the desired storage duration. Normal maintenance requires routine subculturing at standard conditions. Slow-growth storage uses reduced temperatures and osmotic agents to extend the time between subcultures for medium-term preservation. Cryopreservation provides long-term storage in liquid nitrogen. Synthetic seeds encapsulate embryos for short-term storage and easier handling. Together, these techniques provide a comprehensive system for protecting and maintaining plant genetic resources ex situ.

**246.** (B) It neutralizes negative charges on the plasma membrane to allow closer contact.
Protoplasts naturally carry a negative surface charge that causes them to repel each other. During chemical fusion, a high pH environment combined with calcium ions is used to overcome this repulsion. The positive calcium ions help to neutralize the negative charges on the plasma membranes. At the same time, the high pH alters the membrane's physical properties, allowing adjacent protoplasts to come into intimate contact and eventually merge their lipid bilayers.

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enzyme in the nervous system
(B) By binding to midgut receptors to form pores and cause cell lysis
(C) By disrupting chitin synthesis during the insect molting stage
(D) By degrading the host plant DNA ingested by the feeding insect

2011

**10.** Match the non-Bt insect resistance strategies in List I with their primary mechanism or target in List II.
List I: (Resistance Strategy)
P. Protease inhibitors
Q. Plant lectins (GNA)
R. A-amylase inhibitors
S. Vegetative insecticidal proteins (Vip)
List II: (Mechanism/Target)
1. Bind to carbohydrates in the insect gut
2. Disrupt carbohydrate digestion
3. Expressed during vegetative growth, pore-forming
4. Interfere with protein digestion
(A) P-IV, Q-I, R-II, S-III
(B) P-I, Q-IV, R-III, S-II
(C) P-IV, Q-II, R-I, S-III
(D) P-II, Q-III, R-IV, S-I

2004

**11.** Pathogen-derived resistance (PDR) against plant viruses often employs Coat Protein-Mediated Resistance (CPMR). What is the general outcome of CPMR?
(A) The transgenic plant delays or attenuates viral disease symptoms.
(B) The plant produces antibodies against the viral coat protein.
(C) The viral RNA is immediately reverse-transcribed into plant DNA.
(D) The plant coat protein degrades the viral replicase enzyme.

2024

**12.** Which of the following overexpressed Pathogenesis-Related (PR) proteins specifically degrades fungal cell walls?
(A) NPR1 regulatory protein
(B) Sarcotoxin peptides
(C) B-1,3-glucanase
(D) Cecropin peptides

2002

**13.** In genetic engineering for abiotic stress tolerance, the P5CS gene is frequently utilized to overproduce which osmoprotectant?
(A) Glycine βine
(B) Trehalose
(C) Mannitol
(D) Proline

2012

**14.** Which enzyme is targeted and mutated in transgenic crops to confer resistance to the herbicide glyphosate?
(A) Phosphinothricin acetyltransferase (PAT)
(B) Glutamine synthetase enzyme
(C) 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)
(D) Acetolactate synthase (ALS) enzyme

2013

**15.** The Flavr Savr tomato achieved delayed fruit ripening through the antisense suppression of which enzyme?
(A) ACC deaminase
(B) Polygalacturonase (PG)
(C) ACC synthase
(D) ACC oxidase

2004

**16.** Golden Rice was genetically engineered to combat Vitamin A deficiency by expressing the psy and crtI genes. What do these genes facilitate?
(A) The accumulation of iron in the aleurone layer
(B) The synthesis of essential amino acids in the embryo
(C) The breakdown of phytic acid in the seed
(D) The synthesis of β-carotene in the endosperm

2010

**17.** To decrease the levels of polyunsaturated fatty acids and increase oleic acid in seed oil, researchers commonly silence which genes?
(A) FAD2 and FAD3
(B) ACC and PG
(C) EPSPS and GOX
(D) psy and crtI

2016

**18.** Which of the following is a significant advantage of plastid (chloroplast) transformation over nuclear transformation?
(A) It results in Mendelian inheritance of the transgene.
(B) It allows for transgene integration via non-homologous joining.
(C) It reduces gene flow risks via maternal inheritance of the transgene.
(D) It restricts transgene expression solely to the root tissues.

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2010

**19.** Transcriptional Gene Silencing (TGS) in transgenic plants is most frequently associated with which molecular event?
(A) Hypermethylation of the transgene promoter
(B) Rapid degradation of mRNA in the cytoplasm
(C) Inefficient translation by the plant ribosomes
(D) Alternative splicing of the transgenic pre-mRNA

2022

**20.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): The Cre-loxP system can be used to generate marker-free transgenic plants.
Reason (R): Cre is a site-specific recombinase that precisely excises DNA sequences flanked by two identically oriented loxP sites.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**21.** In the Indian biosafety regulatory framework, which body is responsible for granting approval for the large-scale commercial release of Genetically Modified (GM) crops?
(A) Institutional Biosafety Committee (IBSC)
(B) Genetic Engineering Appraisal Committee (GEAC)
(C) Review Committee on Genetic Manipulation (RCGM)
(D) Department of Biotechnology (DBT)

2011

**22.** The concept of "substantial equivalence" in GMO risk assessment implies that:
(A) The GM crop must possess identical DNA to the parental crop.
(B) The GM crop must yield the same as the conventional variety.
(C) Compositionally and nutritionally similar crops are safety-equivalent.
(D) The GM crop and wild relatives must be unable to cross-pollinate.

2023

**23.** Subtractive hybridization is an advantageous technique primarily for which of the following applications?
(A) Transferring whole chromosomes between species
(B) Generating specific mutations within a target promoter
(C) Multiplexing PCR for several transgene markers
(D) Identifying genes differentially expressed between mRNA populations

2003

**24.** Which vir gene product acts as an endonuclease to nick the Agrobacterium Ti plasmid at the T-DNA borders?
(A) VirD2
(B) VirA
(C) VirG
(D) VirE2

NODIA

2002

**25.** What is the defining characteristic of a "disarmed" Ti plasmid used in plant genetic engineering?
(A) It lacks the origin of replication.
(B) It lacks tumor-forming oncogenes.
(C) It lacks left and right T-DNA borders.
(D) It lacks the vir regulon entirely.

2002

**26.** Which chemical agent is routinely used to facilitate direct DNA uptake in protoplast transformation?
(A) Agrobacterium exudate
(B) Polyethylene glycol (PEG)
(C) Acetosyringone inducer
(D) Cellulase enzyme

2004

**27.** The maize ubiquitin-1 promoter is commonly selected for driving transgene expression in which group of plants?
(A) Monocotyledonous plants
(B) Dicotyledonous plants
(C) Gymnosperms
(D) Bryophytes

2024

**28.** In the context of positive selection systems, the pmi gene allows transgenic plant cells to utilize which sugar as a sole carbon source?
(A) Glucose
(B) Sucrose
(C) Mannose
(D) Galactose

2002

**29.** Southern blot hybridization involves the transfer of which molecule to a membrane for subsequent probe hybridization?
(A) RNA
(B) Protein
(C) Carbohydrate
(D) DNA

2014

**30.** Consider the following statements regarding Quantitative Real-Time PCR (qRT-PCR) in transgene analysis:
I. It measures the abundance of a specific mRNA transcript.
II. It requires the conversion of RNA to cDNA prior to amplification.
III. It quantifies the amount of transgenic protein produced.
Which of the above statement(s) is/are correct?
(A) I and III
(B) II and III
(C) I and II
(D) I, II and III

2015

**31.** The refuge crop management strategy is deployed primarily to delay the evolution of resistance in insect populations against which type of transgenic crop?
(A) Bt toxin-expressing crops
(B) Herbicide-tolerant crops
(C) Virus-resistant crops
(D) Golden Rice

2017

**32.** Which non-Bt strategy utilizes carbohydrate-binding proteins to cause insect toxicity?
(A) Protease inhibitors
(B) A-amylase inhibitors
(C) Plant lectins
(D) Vegetative insecticidal proteins

2002

**33.** RNA interference (RNAi) provides virus resistance in transgenic plants by:
(A) Producing coat proteins that encapsulate viral RNA
(B) Mutating the viral DNA genome directly
(C) Inhibiting viral entry into the plant cell wall
(D) Triggering sequence-specific viral RNA degradation

2018

**34.** Phytoalexins are considered an important component of plant defense. What are they?
(A) Large pre-formed structural proteins in the cell wall
(B) Enzymes that degrade bacterial DNA
(C) Antimicrobial compounds synthesized de novo after infection
(D) Transcription factors that activate PR genes

2006

**35.** The transcription factor DREB1/CBF is heavily involved in regulating gene networks that confer tolerance to which type of stress?
(A) Pathogenic fungi
(B) Cold and drought stress
(C) Viral infections
(D) Herbicide application

2014

**36.** Glufosinate herbicide resistance in transgenic crops is typically achieved by introducing which gene?
(A) bxn
(B) EPSPS
(C) GOX
(D) bar or pat

2008

**37.** Which strategy involves downregulating ACC oxidase to extend the shelf-life of fruits?
(A) Preventing the conversion of ACC to ethylene
(B) Increasing the rate of ethylene synthesis
(C) Increasing polygalacturonase enzyme activity
(D) Promoting seed germination efficiency

2017

**38.** The introgression of the ferritin gene into crop plants is a metabolic engineering approach aimed primarily at:
(A) Fortifying the crop with iron
(B) Enhancing β-carotene levels
(C) Increasing protein content
(D) Reducing phytic acid accumulation

2010

**39.** To decrease the levels of polyunsaturated fatty acids and increase oleic acid in seed oil, scientists introduced a gene encoding a specific thioesterase from which organism?
(A) California bay laurel
(B) Bacillus thuringiensis
(C) Agrobacterium tumefaciens
(D) Escherichia coli

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2004

**40.** Select the correct sequence of steps involved in generating marker-free plants via a site-specific recombinase system:
(A) Excision of the marker gene → Introduction of recombinase → Recombination at specific sites → Recovery of marker-free progeny
(B) Introduction of recombinase → Recombination at specific sites → Excision of the marker gene → Recovery of marker-free progeny
(C) Recombination at specific sites → Excision of the marker gene → Introduction of recombinase → Recovery of marker-free progeny
(D) Recovery of marker-free progeny → Introduction of recombinase → Recombination at specific sites → Excision of the marker gene

2005

**41.** Transgene integration into the plastid genome occurs predominantly via which mechanism?
(A) Non-homologous end joining
(B) Agrobacterium vir-mediated integration
(C) Homologous recombination
(D) Transposon tagging

2001

**42.** Post-Transcriptional Gene Silencing (PTGS) can be minimized in transgenic plants by incorporating which DNA elements into the transformation vector?
(A) Enhancer traps
(B) Multiple cloning sites
(C) Matrix Attachment Regions (MARs)
(D) Constitutive promoters

2016

**43.** The FLP-FRT excision system utilized for marker removal is originally derived from which organism?
(A) Bacteriophage P1
(B) Agrobacterium tumefaciens
(C) Cauliflower mosaic virus
(D) Saccharomyces cerevisiae

2006

**44.** Confined field trials (BRL-I and BRL-II) in India are mandatory to evaluate which aspect of a genetically engineered crop?
(A) Only the nutritional profile of the crop
(B) Laboratory-scale expression levels of the transgene
(C) The efficiency of plasmid construction
(D) Agronomic performance and environmental safety

2016

**45.** Which regulatory body is responsible for monitoring the safety of ongoing research activities in biotechnology at the institutional level in India?
(A) GEAC
(B) IBSC
(C) RCGM
(D) NBPGR

2002

**46.** Transposon tagging relies on the principle of:
(A) Utilizing radioactive labels to identify localized chromosomes
(B) Coating microcarriers with DNA for high-velocity bombardment
(C) Hybridizing cDNA probes to specific mRNA transcript populations
(D) Inserting a known mobile element to tag a gene of interest

2013

**47.** Which components of the Agrobacterium vir regulon form a two-component regulatory system that senses plant wound signals?
(A) VirA and VirG
(B) VirD1 and VirD2
(C) VirE1 and VirE2
(D) VirB and VirC

NODIA

2010

**48.** In a binary vector system, the helper plasmid functions to provide:
(A) The origin of replication for E. coli
(B) The left and right borders of T-DNA
(C) The vir genes in trans for T-DNA transfer
(D) The selectable marker for plant cells

2004

**49.** The pollen-tube pathway method of direct gene transfer involves applying DNA to which specific plant tissue?
(A) Stigmas after pollination
(B) Excised root tips
(C) Protoplasts in suspension
(D) Leaf explants

2017

**50.** Consider the following statements regarding the use of reporter genes:
I. They confirm successful gene integration and expression.
II. The gusA gene produces a blue precipitate when supplied with X-Gluc.

III. The gfp gene requires a specific exogenous substrate to emit fluorescence.
IV. The luc gene codes for luciferase which requires luciferin.

Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II and III
(C) III only
(D) I, II and IV

2006

**51.** Which plant promoter would be most appropriate to express a transgene exclusively in the seeds of a crop?
(A) Napin promoter
(B) CaMV 35S
(C) Rice actin-1 promoter
(D) Rubisco small subunit promoter

2015

**52.** What is the fundamental target detected by a Western blot assay in transgene expression analysis?
(A) The integrated DNA sequence
(B) The transcribed mRNA
(C) The specific translated protein
(D) The lipid profile modifications

2001

**53.** If a researcher wishes to determine whether an inserted transgene exists as a single copy or multiple copies, which technique is most analytically robust?
(A) Northern blot
(B) RT-PCR
(C) Southern blot
(D) Microinjection

2007

**54.** What is the major limitation of using constitutive promoters to drive resistance genes in transgenic crops?
(A) They only function in dicots.
(B) They are rapidly silenced by RNA interference.
(C) They require chemical induction to function.
(D) They can lead to a metabolic penalty.

2017

**55.** Which of the following is an example of an inducible promoter?
(A) CaMV 35S
(B) Cold-responsive promoter
(C) Maize ubiquitin-1
(D) Phaseolin promoter

2008

**56.** The bar gene from Streptomyces hygroscopicus is widely used as a plant selectable marker. How does it function?
(A) It degrades kanamycin.
(B) It provides resistance to glyphosate.
(C) It acetylates phosphinothricin to detoxify it.
(D) It converts mannose to a usable carbohydrate.

2011

**57.** Which of the following accurately describes the role of the VirE2 protein in Agrobacterium-mediated transformation?
(A) It nicks the double-stranded DNA at the conserved borders.
(B) It acts as a sensor kinase in the bacterial cell membrane.
(C) It is a structural component of the bacterial T-pilus.
(D) It is a single-stranded DNA binding protein for T-strand protection.

2009

**58.** The phenomenon of "gene stacking" or "pyramiding" in Bt crops refers to:
(A) Using multiple distinct Bt genes to delay resistance
(B) Planting rows of Bt crops interspersed with non-Bt crops
(C) Increasing the copy number of a single Bt gene at one locus
(D) Combining Bt resistance with viral resistance using RNAi

2010

**59.** Replicase-mediated resistance against plant viruses relies on the expression of:
(A) A fungal β-glucanase
(B) A modified viral replicase gene in the host
(C) A bacterial RNase
(D) A plant specific protease inhibitor

2014

**60.** Arrange the following steps of RNAi-mediated virus resistance in the correct order:
1. Dicer cleaves dsRNA into siRNAs.
2. Transcription of a hairpin RNA construct in the plant.
3. Target viral RNA is degraded.
4. siRNAs are incorporated into the RISC complex.

(A) 2, 1, 4, 3
(B) 1, 2, 4, 3
(C) 2, 4, 1, 3
(D) 4, 2, 1, 3

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# CHAPTER 11
# Plant Genetic Engineering

2008

**1.** Which of the following best describes map-based cloning in plant biotechnology?
(A) Isolating a gene based on its genetic position and linkage to markers
(B) Cloning a gene based on its expression profile in various plant tissues
(C) Identifying a gene through homologous sequences in other species
(D) Using transposons to recover specific flanking genomic regions

2008

**2.** What is the primary role of acetosyringone during the Agrobacterium-mediated transformation process?
(A) It acts as a primary selectable marker for newly transformed plant cells.
(B) It facilitates the structural formation of the conserved T-DNA borders.
(C) It induces the expression of the Agrobacterium vir regulon.
(D) It protects the transferred DNA from degradation by cellular nucleases.

2004

**3.** In the context of Agrobacterium vectors, how does a binary vector system fundamentally differ from a co-integrative vector system?
(A) It houses T-DNA and vir genes on two separate replicons.
(B) It contains the vir genes and T-DNA on a single large plasmid.
(C) It requires homologous recombination between the target plasmids.
(D) It lacks a selectable marker for identifying transformed cells.

2004

**4.** Which parameter is considered most critical for optimizing direct gene transfer via particle bombardment (biolistics)?
(A) The concentration of acetosyringone in the shooting medium
(B) The duration of protoplast incubation in PEG
(C) The size of the T-DNA left and right borders
(D) The helium gas pressure and target distance

2004

**5.** Which of the following is considered a strictly constitutive promoter used in plant genetic engineering?
(A) Seed-specific phaseolin promoter
(B) Cauliflower Mosaic Virus (CaMV) 35S promoter
(C) Wound-inducible defense promoter
(D) Cold-responsive stress promoter

2004

**6.** The plant selectable marker gene nptII confers resistance to which of the following antibiotics?
(A) Hygromycin
(B) Phosphinothricin
(C) Kanamycin
(D) Spectinomycin

NODIA

2012

**7.** What is the primary purpose of Thermal Asymmetric Interlaced PCR (TAIL-PCR) in transgenic plant analysis?
(A) To quantify the exact level of transgene RNA transcript
(B) To measure the concentration of the translated transgenic protein
(C) To detect single nucleotide polymorphisms within the transgene
(D) To isolate unknown genomic DNA flanking the transgene insertion site

2017

**8.** For detecting the stable integration of a transgene into the plant genome, which molecular technique is considered the gold standard?
(A) Southern blot hybridization
(B) Northern blot analysis
(C) Western blot analysis
(D) Enzyme-Linked Immunosorbent Assay (ELISA)

2014

**9.** How do Bacillus thuringiensis (Bt) Cry proteins primarily exert their toxicity on susceptible insect pests?
(A) By inhibiting the acetylcholinesterase

genes
(C) Prevent cross-pollination with wild species
(D) Generate marker-free plants via gene separation

2006

**73.** The site-specific recombinase "R" which recognizes "RS" sites was originally isolated from which yeast species?
(A) Saccharomyces cerevisiae
(B) Zygosaccharomyces rouxii
(C) Pichia pastoris
(D) Candida albicans

2021

**74.** Under the Indian Biosafety Regulatory Framework, the Review Committee on Genetic Manipulation (RCGM) functions under which ministry?
(A) Ministry of Science and Technology
(B) Ministry of Environment, Forest and Climate Change (MoEFCC)
(C) Ministry of Agriculture and Farmers Welfare
(D) Ministry of Health and Family Welfare

2005

**75.** A key concern regarding "gene flow" in GMO risk assessment is the potential creation of "superweeds." This occurs when:
(A) A Bt gene causes weeds to grow excessively tall.
(B) The transgenic crop outcompates native flora in a forest ecosystem.
(C) Insects develop resistance to multiple herbicides.
(D) Herbicide tolerance is transferred to wild weeds.

2002

**76.** Which of the following is evaluated to assess the potential allergenicity of a newly introduced transgenic protein?
(A) Amino acid sequence homology to known allergens
(B) The melting temperature of the transgene DNA
(C) The number of introns in the transgene
(D) The size of the vector plasmid used for transformation

2004

**77.** The construction of a genomic library requires the initial fragmentation of genomic DNA, which is most commonly achieved using:
(A) DNA ligase
(B) Reverse transcriptase
(C) Taq polymerase
(D) Restriction endonucleases

2024

**78.** In cDNA library construction, which enzyme is essential for synthesizing the first strand of DNA from an mRNA template?
(A) Reverse transcriptase
(B) DNA polymerase I
(C) RNase H
(D) T4 DNA ligase

2019

**79.** Which component of the Agrobacterium Ti plasmid contains the genes required for the synthesis of opines?
(A) The vir region
(B) The origin of replication
(C) The chv genes on the bacterial chromosome
(D) The T-DNA region

2003

**80.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): The use of a binary vector system is generally preferred over a co-integrative system for plant transformation.
Reason (R): Binary vectors are smaller, easier to manipulate in E. coli, and do not rely on homologous recombination for T-DNA maintenance.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**81.** Electroporation facilitates direct gene transfer by:
(A) Using a fine glass needle to inject DNA
(B) Coating gold particles with DNA and shooting them
(C) Creating transient membrane pores via electricity
(D) Utilizing chemical porins isolated from bacteria

2022

**82.** Which of the following represents an inherent disadvantage of the microinjection technique for plant transformation?
(A) It has an extremely low transformation frequency per cell injected.

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2001

**148.** Why is marker-free transgenic plant development considered highly desirable?
(A) It addresses regulatory safety concerns.
(B) It increases the transformation efficiency.
(C) It allows the plant to grow without fertilizer.
(D) It makes the plant immune to all viral diseases.

2024

**149.** Transcriptional Gene Silencing (TGS) prevents the transcription of a transgene. Which chromosomal modification is an epigenetic hallmark of TGS?
(A) Histone acetylation
(B) DNA hypomethylation
(C) Telomere shortening
(D) Cytosine methylation of the promoter

2008

**150.** Arrange the regulatory steps for the approval of a new GM crop in India in the correct chronological order:
1. Approval by GEAC for commercial release
2. Evaluation by IBSC at the institutional level
3. Review and approval by RCGM for confined field trials

(A) 2, 3, 1
(B) 1, 2, 3
(C) 3, 2, 1
(D) 2, 1, 3

2018

**151.** Which of the following is evaluated to assess the potential allergenicity of a newly introduced transgenic protein?
(A) Evaluating protein resemblance to allergens
(B) Checking if the GM plant causes allergies in insects
(C) Ensuring the plant does not produce pollen
(D) Testing the herbicide resistance of the crop

2022

**152.** In a cDNA library, the sequences represented do not include:
(A) Exons
(B) 5' untranslated regions (partially)
(C) Promoter regions and introns
(D) 3' untranslated regions

2024

**153.** The Agrobacterium virB operon encodes proteins that function to:
(A) Sense plant phenolic signals
(B) Synthesize plant hormones
(C) Form a secretion channel for T-complex export
(D) Replicate the Ti plasmid

2004

**154.** What is the role of the left and right border sequences of the T-DNA?
(A) They encode the vir genes.
(B) They define the segment to be transferred.
(C) They are origins of replication.
(D) They synthesize opines.

2012

**155.** Which direct gene transfer method utilizes tungsten or gold particles?
(A) Electroporation
(B) Biolistics (Particle bombardment)
(C) Microinjection
(D) PEG-mediated transformation

2010

**156.** The napin promoter is well-known for driving high-level transgene expression in which plant organ?
(A) Roots
(B) Leaves
(C) Flowers
(D) Seeds

NODIA

2008

**157.** Which of the following is often used as a screenable marker rather than a selectable marker?
(A) nptII
(B) hpt
(C) gusA
(D) bar

2006

**158.** TAIL-PCR is particularly advantageous because it requires:
(A) One specific primer and a degenerate primer
(B) No primers
(C) Complete knowledge of the whole genome sequence
(D) Radioactive labeling

2022

**159.** When analyzing RNA by Northern blot, which step ensures the RNA molecules are separated by size?
(A) Hybridization
(B) Capillary transfer
(C) Gel electrophoresis
(D) Autoradiography

2016

**160.** Match the molecular analysis techniques in List I with their primary output or metric in List II.

2016

**93.** A primary limitation of using pathogen-derived resistance (PDR) via coat protein is:
(A) It is often narrowly specific to the source virus.
(B) It causes severe stunting in the host plant.
(C) It rapidly breaks down in the presence of herbicides.
(D) It requires constant chemical induction.

2009

**94.** Cecropins, utilized in transgenic plants for disease resistance, are a class of antimicrobial peptides originally isolated from:
(A) Frogs
(B) Soil bacteria
(C) Fungi
(D) Insects

2002

**95.** The NPR1 protein acts as a master regulator in the plant defense system by modulating the expression of:
(A) Pathogenesis-Related (PR) genes
(B) ACC synthase genes
(C) EPSPS genes
(D) DREB transcription factors

2022

**96.** Late Embryogenesis Abundant (LEA) proteins are hypothesized to protect plants during drought stress by:
(A) Acting as chaperones to prevent denaturation
(B) Actively transporting water into the cell
(C) Closing the stomata permanently
(D) Degrading abscisic acid

2012

**97.** The introduction of the glyphosate oxidoreductase (GOX) gene confers herbicide tolerance by:
(A) Preventing glyphosate from entering the cell
(B) Degrading glyphosate into harmless products
(C) Mutating the target enzyme
(D) Overproducing the target enzyme

2009

**98.** Antisense RNA technology involves introducing a gene construct that produces an RNA transcript which is:
(A) Identical to the target mRNA
(B) A double-stranded hairpin
(C) Complementary to the target endogenous mRNA
(D) Lacking a poly-A tail

NODIA

2007

**99.** Which of the following represents a metabolic engineering approach to reduce anti-nutritional factors in feed crops?
(A) Overexpression of ferritin
(B) Fungal phytase expression to break phytic acid
(C) Silencing of FAD2
(D) Introduction of the psy gene

2010

**100.** Match the selectable marker genes in List I with their respective selecting agents in List II.
List I: (Selectable Marker Gene)
P. hpt
Q. bar
R. nptII
S. pmi
List II: (Selecting Agent)
1. Phosphinothricin
2. Mannose
3. Kanamycin
4. Hygromycin
(A) P-IV, Q-I, R-III, S-II
(B) P-I, Q-IV, R-II, S-III
(C) P-IV, Q-III, R-I, S-II
(D) P-III, Q-I, R-IV, S-II

2025

**101.** Which modification is most relevant for increasing the thermostability and shelf-life of frying oils derived from transgenic plants?
(A) Reducing polyunsaturates by silencing desaturases
(B) Increasing linolenic acid content
(C) Decreasing oleic acid content
(D) Introducing the EPSPS gene

2009

**102.** In plastid transformation, targeting the transgene to specific regions of the chloroplast genome is achieved by flanking the transgene with:
(A) Left and right T-DNA borders
(B) Viral long terminal repeats
(C) Sequences homologous to the chloroplast genome
(D) Matrix attachment regions

2013

**103.** Multi-gene operon expression is a distinct feature of which transformation system?
(A) Nuclear transformation via Agrobacterium
(B) Biolistics in the nucleus
(C) Plastid transformation
(D) Microinjection

(C) A is true but R is false.
(D) A is false but R is true.

2015

**171.** Which mechanism is primarily responsible for Post-Transcriptional Gene Silencing (PTGS) in plants?
(A) siRNA production and mRNA cleavage
(B) RNA-directed DNA methylation (RdDM)
(C) The removal of the poly-A tail during transcription
(D) Ribosome stalling at the start codon

2003

**172.** The FLP-FRT recombination system utilizes the FLP recombinase which specifically recognizes:
(A) 34-bp loxP sites
(B) 34-bp FRT sites
(C) 25-bp T-DNA borders
(D) 50-bp RS sites

2015

**173.** What is the main function of the Genetic Engineering Appraisal Committee (GEAC) in India?
(A) Environmental appraisal of large-scale GMO use
(B) To conduct preliminary laboratory research on GMOs
(C) To fund biotechnology research projects
(D) To issue patents for transgenic crops

2025

**174.** In the context of risk assessment, analyzing "gene flow" is critical to determine the likelihood of:
(A) Transgene movement to wild relatives
(B) Genes moving from the chloroplast to the nucleus
(C) The GM crop becoming toxic to humans
(D) The rapid mutation of the inserted transgene

2013

**175.** Which technique separates complex mRNA mixtures into distinct bands to identify genes expressed under specific conditions?
(A) Map-based cloning
(B) Transposon tagging
(C) Differential display
(D) Southern blotting

2003

**176.** Which Vir protein binds to the "overdrive" sequence to enhance the efficiency of T-strand production?
(A) VirA
(B) VirC1
(C) VirG
(D) VirE2

2023

**177.** A co-integrative vector system requires a region of homology between the intermediate vector and the Ti plasmid. What is the purpose of this homology?
(A) To facilitate recombination to join plasmids
(B) To initiate DNA replication
(C) To synthesize acetosyringone
(D) To express the selectable marker

2019

**178.** PEG-mediated transformation is typically applied to which biological target?
(A) Intact leaves
(B) Seeds
(C) Protoplasts
(D) Pollen grains

2023

**179.** The rice actin-1 promoter is preferred over the CaMV 35S promoter for stable, high-level expression in:
(A) Monocot species
(B) Dicot species
(C) Fungal species
(D) Bacterial species

2013

**180.** Consider the following statements regarding reporter genes:
I. The gfp gene allows non-destructive, in vivo detection.
II. The gusA (GUS) assay is typically destructive to the tissue.
III. The luc gene product requires luciferin to emit light.
IV. Reporter genes confer herbicide resistance.
V. They are used to visually verify successful transformation and analyze promoter activity.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) II, III, IV and V
(C) I, II, IV and V
(D) I, III, IV and V

2021

**181.** In Southern blot hybridization, the DNA must be denatured into single strands before transfer to the membrane. Why?
(A) To facilitate labeled nucleic acid probe binding
(B) To allow the restriction enzymes to cut

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2018

**115.** Quantitative Real-Time PCR (qRT-PCR) measures the accumulation of PCR product during which phase of the reaction?
(A) The plateau phase
(B) The exponential phase
(C) The denaturation phase
(D) The final cooling phase

2016

**116.** In the mode of action of Cry toxins, which domain of the protein is primarily responsible for binding to the specific receptors on the insect midgut epithelium?
(A) Domain I
(B) Domain II
(C) Domain III
(D) The C-terminal protoxin tail

2024

**117.** What distinguishes Vegetative Insecticidal Proteins (Vip) from Cry proteins in terms of their physical presentation in the bacterium?
(A) Vip are secreted during growth; Cry are crystalline at sporulation.
(B) Vip are cell-wall embedded; Cry are secreted into the medium.
(C) Vip are RNA-based signals; Cry are small peptide-based toxins.
(D) Vip only function biologically when Cry proteins are present.

2023

**118.** Which transgenic approach employs double-stranded RNA (dsRNA) designed against an essential insect pest gene to cause mortality?
(A) Coat Protein-Mediated Resistance (CPMR)
(B) Pathogen-Derived Resistance (PDR)
(C) EPSPS overexpression
(D) Host-Induced Gene Silencing (HIGS) via RNAi

2005

**119.** Sarcotoxins are a type of antimicrobial peptide utilized in genetic engineering. What is their biological origin?
(A) Plant roots
(B) The flesh fly (Sarcophaga peregrina)
(C) Soil-borne fungi
(D) Marine algae

2025

**120.** Consider the following statements regarding abiotic stress tolerance engineering:
I. DREB1/CBF genes encode transcription factors.
II. LEA proteins are mostly hydrophobic and localized in the membrane.
III. Proline accumulation helps maintain cellular osmolarity.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II only
(C) I and III
(D) II and III

NODIA

2016

**121.** The bromoxynil resistance gene (bxn) originates from which organism?
(A) Klebsiella pneumoniae ozaenae
(B) Agrobacterium tumefaciens
(C) Bacillus thuringiensis
(D) Streptomyces viridochromogenes

2013

**122.** ACC synthase catalyzes which specific reaction in the ethylene biosynthesis pathway?
(A) The conversion of ACC to ethylene
(B) The breakdown of ethylene into inactive metabolites
(C) SAM to 1-aminocyclopropane-1-carboxylic acid
(D) The conversion of methionine to SAM

2008

**123.** The crtI gene used in Golden Rice development encodes which enzyme?
(A) Phytoene synthase
(B) Lycopene β-cyclase
(C) ACC oxidase
(D) Phytoene desaturase

2024

**124.** Modifying fatty acid composition by targeting desaturase genes predominantly affects:
(A) The length of the carbon chain in fatty acids
(B) The attachment of fatty acids to the glycerol backbone
(C) The number of double bonds in the carbon chain
(D) The breakdown of triglycerides during germination

2022

**125.** Unlike nuclear transformation, chloroplast transformation is generally exempt from which of the following phenomena?
(A) High protein accumulation
(B) Homologous recombination
(C) Gene silencing
(D) Selection marker usage

2013

**104.** Matrix Attachment Regions (MARs) help to stabilize transgene expression primarily by:
(A) Providing strong enhancer sequences
(B) Anchoring chromatin to shield from silencing
(C) Degrading small interfering RNAs
(D) Facilitating the export of mRNA to the cytoplasm

2007

**105.** In the Cre-loxP system, if the two loxP sites flanking a marker gene are in the same orientation (direct repeats), the action of the Cre recombinase will result in:
(A) Inversion of the marker gene
(B) Duplication of the marker gene
(C) Excision of the marker gene
(D) Silencing of the marker gene via methylation

2003

**106.** The Institutional Biosafety Committee (IBSC) must include a nominee from which regulatory body to ensure compliance?
(A) GEAC
(B) DBT
(C) ICAR
(D) NBPGR

2007

**107.** Which of the following parameters is evaluated during confined field trials (BRL-I) of a GM crop?
(A) Complete elimination of non-target insects
(B) Phenotypic traits and potential for weediness
(C) The chemical sequence of the Agrobacterium vector
(D) Cost-benefit analysis of commercialization

2006

**108.** Differential display is a transcriptomics technique used to:
(A) Sequence the entire genome of a plant
(B) Identify differentially expressed mRNAs
(C) Visualize the physical location of a gene on a chromosome
(D) Transfer proteins onto a nitrocellulose membrane

2019

**109.** Which compound, secreted by wounded plant cells, serves as a powerful inducer of the Agrobacterium vir genes?
(A) Acetosyringone
(B) Salicylic acid
(C) Jasmonic acid
(D) Ethylene

2007

**110.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Agrobacterium tumefaciens cannot be used to transform monocotyledonous plants under any circumstances.
Reason (R): Monocots do not produce the specific phenolic compounds required to induce the vir regulon.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**111.** Which method of direct gene transfer is highly dependent on the transient enzymatic removal of the plant cell wall?
(A) Electroporation of protoplasts
(B) Particle bombardment
(C) Pollen-tube pathway
(D) Microinjection of zygotes

2021

**112.** The seed-specific phaseolin promoter is originally derived from which plant?
(A) Zea mays (Maize)
(B) Oryza sativa (Rice)
(C) Phaseolus vulgaris (Common bean)
(D) Arabidopsis thaliana

2009

**113.** In positive selection systems (like pmi), transformed cells are selected because they:
(A) Are immune to a toxic antibiotic
(B) Are resistant to a lethal herbicide
(C) Produce a visible colored pigment
(D) Can metabolize unique carbon sources

2025

**114.** PCR-based confirmation of a transgene can occasionally yield false positives. What is a common cause of this in Agrobacterium-mediated transformation?
(A) Residual Agrobacterium cells containing vectors
(B) Cross-amplification with plant genomic DNA
(C) Degradation of the template DNA
(D) Insufficient primer annealing temperature

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(D) Oxidation

2013

**137.** Which of the following PCR techniques involves a series of nested primers and varying annealing temperatures to amplify unknown DNA flanking a known sequence?
(A) Real-Time PCR
(B) Reverse Transcriptase PCR
(C) TAIL-PCR
(D) Multiplex PCR

2011

**138.** In a Western blot protocol, what is the purpose of using non-fat dry milk or bovine serum albumin (BSA) after the protein transfer?
(A) To provide nutrients for the antibody
(B) To denature the proteins on the membrane
(C) To block non-specific binding sites on the membrane
(D) To visualize the protein bands directly

2006

**139.** Gene pyramiding in insect-resistant crops provides a durable resistance strategy because:
(A) It decreases the overall expression level of the toxins.
(B) It requires simultaneous developer mutations.
(C) It increases the crop yield independently of pest pressure.
(D) It completely eradicates the insect population in one generation.

2010

**140.** Consider the following statements regarding non-Bt insect resistance:
I. Protease inhibitors affect insect protein digestion.
II. A-amylase inhibitors are highly effective against lipid-digesting pests.
III. Plant lectins bind to the midgut epithelial glycoproteins.
IV. Vip proteins do not share sequence homology with Cry proteins.
Which of the above statement(s) is/are INCORRECT?
(A) II only
(B) I and II
(C) III and IV
(D) II and IV

2022

**141.** Coat Protein-Mediated Resistance (CPMR) typically confers resistance to:
(A) The specific source virus and related strains
(B) Fungal pathogens
(C) Bacterial wilt
(D) Root-knot nematodes

2019

**142.** Chitinases are extensively utilized in transgenic plants to provide resistance against which class of pathogens?
(A) Viruses
(B) Gram-positive bacteria
(C) Fungi
(D) Nematodes

2024

**143.** Which type of abiotic stress is specifically targeted by the overexpression of the choline monooxygenase gene?
(A) Heavy metal toxicity
(B) Heat shock
(C) UV radiation
(D) Salinity or drought osmotic stress

2013

**144.** Which enzyme is responsible for the detoxification of the herbicide glufosinate in resistant transgenic plants?
(A) EPSPS
(B) GOX
(C) Phosphinothricin acetyltransferase (PAT)
(D) Acetolactate synthase

NODIA

2019

**145.** The antisense ACC synthase technology for delayed ripening was first successfully demonstrated in which fruit?
(A) Banana
(B) Cantaloupe melon
(C) Pineapple
(D) Strawberry

2007

**146.** Which fundamental biochemical pathway was genetically modified to produce Golden Rice?
(A) The carotenoid biosynthetic pathway
(B) The shikimate pathway
(C) The anthocyanin pathway
(D) The glycolysis pathway

2011

**147.** Altering the saturation levels of plant oils involves modifying the activity of which class of enzymes?
(A) Thioesterases
(B) Desaturases
(C) Lipases
(D) Hydroxylases

2017

**126.** Homology-Dependent Gene Silencing (HDGS) can manifest as either TGS or PTGS. What triggers HDGS?
(A) The absence of a promoter in the transgene construct
(B) Multiple transgene copies with sequence homology
(C) The use of a tissue-specific promoter
(D) The expression of a single-copy transgene at moderate levels

2006

**127.** In the co-transformation strategy for marker removal, what is the crucial requirement for the marker and the gene of interest?
(A) Integration into the genome at unlinked loci
(B) Location on the exact same T-DNA molecule
(C) Shared usage of the same promoter sequence
(D) Transfer using particle bombardment only

2014

**128.** The Genetic Engineering Appraisal Committee (GEAC) operates under the aegis of which Indian Ministry?
(A) Ministry of Agriculture and Farmers Welfare
(B) Ministry of Science and Technology
(C) Ministry of Environment, Forest and Climate Change
(D) Ministry of Commerce and Industry

2010

**129.** When assessing the effect of a Bt crop on non-target organisms, which of the following is a primary test subject?
(A) The target lepidopteran pest
(B) Beneficial insects like honeybees and beetles
(C) The soil bacteria Agrobacterium tumefaciens
(D) The human consumer

2001

**130.** Select the correct sequence of molecular events during Agrobacterium T-DNA processing:
(A) VirD2 nicks the border → T-strand synthesis → VirE2 binds T-strand → Export via VirB/D4 complex
(B) VirE2 binds T-strand → VirD2 nicks the border → T-strand synthesis → Export via VirB/D4 complex
(C) Export via VirB/D4 complex → VirD2 nicks the border → T-strand synthesis → VirE2 binds T-strand
(D) T-strand synthesis → VirE2 binds T-strand → Export via VirB/D4 complex → VirD2 nicks the border

2004

**131.** Which of the following best defines a "genomic library"?
(A) Cloned fragments representing the whole genome
(B) DNA fragments representing only expressed genes
(C) A digital database of DNA sequences
(D) A set of purified RNA molecules from a specific tissue

2006

**132.** The VirD2 protein remains covalently attached to which end of the T-strand during its transfer to the plant nucleus?
(A) The 3' end
(B) The 5' end
(C) Both ends
(D) It does not attach to the T-strand

2009

**133.** Agrobacterium tumefaciens induces the plant to produce opines, which are primarily used by the bacteria as a source of:
(A) Carbon and nitrogen
(B) Plant hormones
(C) DNA polymerase
(D) Defense against other soil bacteria

2022

**134.** Which physical parameter is generally optimized to improve transformation efficiency in electroporation?
(A) The pressure of the helium gas
(B) The concentration of acetosyringone
(C) The size of the gold microcarriers
(D) Electric field strength and pulse duration

2004

**135.** The CaMV 35S promoter is highly active in dicots. How does its activity generally compare in monocot plants?
(A) It is entirely inactive in monocots.
(B) Its activity is often lower in monocots.
(C) It is equally active in monocots without any modifications.
(D) It functions as a wound-inducible promoter in monocots.

2011

**136.** The hpt gene confers resistance to hygromycin by encoding an enzyme that performs which chemical modification?
(A) Phosphorylation
(B) Acetylation
(C) Methylation

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List I: (Molecular Analysis Technique)
P. Southern blot
Q. Northern blot
R. Western blot
S. qRT-PCR
List II: (Output/Metric)
1. Exact quantification of mRNA copy number
2. Determination of transgene copy number and integration
3. Detection of specific protein size and presence
4. Detection of specific mRNA transcript size and presence

(A) P-II, Q-IV, R-III, S-I
(B) P-I, Q-II, R-IV, S-III
(C) P-II, Q-III, R-IV, S-I
(D) P-IV, Q-II, R-I, S-III

2023

**161.** Resistance management in Bt crops often mandates a "refuge". A refuge consists of:
(A) Areas planted with non-Bt versions of the crop
(B) Areas planted with a different crop species entirely
(C) Areas heavily sprayed with insecticides
(D) Areas where no plants are allowed to grow

2005

**162.** How does the vegetative insecticidal protein (Vip3A) induce mortality in susceptible insects?
(A) By degrading the peritrophic membrane
(B) By inhibiting the insect's respiratory chain
(C) By binding midgut receptors to form pores
(D) By dissolving the insect exoskeleton

2016

**163.** The use of RNA interference (RNAi) against plant viruses typically targets which viral component?
(A) The viral sequence, leading to its degradation
(B) The viral lipid envelope
(C) The viral DNA polymerase
(D) The plant's cell surface receptors

2025

**164.** Antimicrobial peptides (AMPs) like cecropins generally kill bacterial pathogens by:
(A) Mutating bacterial DNA
(B) Disrupting cell membrane integrity
(C) Inhibiting bacterial ribosomes
(D) Chelating essential iron

2025

**165.** The DREB1/CBF transcription factors bind to specific cis-acting elements (DRE/CRT) in the promoters of target genes. What does DRE stand for?
(A) Drought-Responsive Element
(B) DNA-Replication Element
(C) Disease-Resistance Enhancer
(D) Dark-Repressed Element

NODIA

2012

**166.** Herbicide-tolerant crops engineered with the CP4 EPSPS gene are resistant to glyphosate because the CP4 EPSPS enzyme:
(A) Degrades glyphosate rapidly
(B) Pumps glyphosate out of the chloroplast
(C) Converts glyphosate into a useful nutrient
(D) Has a vastly reduced affinity for glyphosate

2018

**167.** Antisense suppression of ACC oxidase in tomatoes results in:
(A) Significantly reduced ethylene and delayed ripening
(B) Immediate fruit drop
(C) Enhanced ethylene production
(D) Increased susceptibility to fungal rots

2015

**168.** To enhance the bioavailable iron content in crops, expressing a ferritin gene is often combined with the expression of which other enzyme to reduce an iron-chelating anti-nutrient?
(A) Phytase
(B) Protease
(C) Amylase
(D) Lipase

2012

**169.** What is the primary purpose of silencing the FAD3 desaturase gene in oilseed crops?
(A) To increase the level of linolenic acid (18:3)
(B) To decrease linolenic acid to increase stability
(C) To increase the level of stearic acid (18:0)
(D) To decrease the total oil content of the seed

2017

**170.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Plastid transformation allows for high-level accumulation of transgenic proteins.
Reason (R): A single plant cell can contain thousands of copies of the plastid genome, resulting in a massive gene copy number.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.

(B) It requires specialized equipment and skilled staff.
(C) It can only be performed on intact, whole plants.
(D) It inevitably causes severe viral infections in the plant.

2023

**83.** The pat gene, used as a selectable marker, confers tolerance to:
(A) Kanamycin
(B) Hygromycin
(C) Phosphinothricin
(D) Glyphosate

2019

**84.** Which of the following statements about the Green Fluorescent Protein (GFP) reporter system is correct?
(A) It is lethal to the plant cells expressing it.
(B) It is visualized non-destructively under UV light.
(C) It requires the addition of luciferin to emit light.
(D) It requires the destruction of the tissue for the assay.

2024

**85.** What is the fundamental difference between Northern blotting and Western blotting?
(A) Northern blotting uses antibodies; Western blotting uses nucleic acid probes.
(B) Northern blotting detects RNA; Western blotting detects proteins.
(C) Northern blotting detects DNA; Western blotting detects RNA.
(D) Northern blotting relies on PCR; Western blotting relies on sequencing.

2008

**86.** In a standard ELISA test used for detecting a transgenic protein, what is typically conjugated to the secondary antibody?
(A) A radioactive isotope
(B) A fluorescent dye
(C) An enzyme producing a colored product
(D) A specific DNA sequence

2024

**87.** The Cry proteins produced by Bacillus thuringiensis are initially synthesized in the bacterium as:
(A) Active toxins
(B) Inactive protoxins
(C) Small interfering RNAs
(D) Soluble membrane receptors

2006

**88.** Which of the following insect orders is primarily targeted by the Cry1 class of Bt toxins?
(A) Lepidoptera
(B) Coleoptera
(C) Diptera
(D) Hemiptera

2011

**89.** Cyt proteins differ from Cry proteins in Bt formulations primarily because Cyt proteins:
(A) Are active against Lepidopteran larvae
(B) Affect midgut cells without specific receptors
(C) Interfere with insect molting directly
(D) Are synthesized during the vegetative growth phase

2007

**90.** Consider the following statements regarding the refuge strategy in Bt crop cultivation:
I. It involves planting a percentage of non-Bt crops near the Bt crops.
II. It aims to maintain a population of susceptible insects.
III. Susceptible insects mate with resistant insects, keeping the resistance allele frequency low.
IV. It utilizes multiple Bt genes simultaneously in the same plant.
V. It is a mandatory requirement in many regulatory frameworks for Bt crops.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2005

**91.** Vegetative insecticidal proteins (Vip) are advantageous in pest management because:
(A) They target the same midgut receptors as Cry proteins, enhancing toxicity.
(B) They bind to different receptors than Cry proteins.
(C) They are expressed only in the roots of the plant.
(D) They act as a physical barrier on the leaf surface.

2024

**92.** The GNA lectin (Galanthus nivalis agglutinin) is primarily toxic to which group of insects?
(A) Chewing insects (e.g., caterpillars)
(B) Sap-sucking insects (e.g., aphids)
(C) Root-boring insects
(D) Pollinators

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(C) To prevent the DNA from degrading
(D) To activate the reporter gene

2008

**182.** ELISA relies on the specific interaction between:
(A) Antigen and antibody
(B) DNA and RNA
(C) Primer and template
(D) Enzyme and RNA

2003

**183.** To prevent the development of resistance in insects, a highly effective strategy is to pyramid a Cry protein with a Vip protein. Why is this effective?
(A) They differ in modes of action and cross-resistance.
(B) They both bind to the exact same receptor, doubling the toxicity.
(C) Vip proteins degrade Cry proteins.
(D) Vip proteins attract predators of the insect.

2003

**184.** Protease inhibitors engineered into plants combat insect pests by:
(A) Inhibiting digestive enzymes to cause starvation
(B) Creating holes in the insect gut
(C) Preventing the insect from recognizing the host plant
(D) Digesting the insect's exoskeleton

2007

**185.** Replicase-mediated resistance relies on the concept of Pathogen-Derived Resistance (PDR). It generally works by:
(A) Expressing mutant replicase to interfere with viruses
(B) Mutating the plant genome
(C) Producing a toxin that kills the viral vector (e.g., aphids)
(D) Enhancing plant cell wall thickness

2004

**186.** B-1,3-glucanase is often co-expressed with chitinase in transgenic plants to enhance resistance against fungi. Why are they synergistic?
(A) They degrade two distinct structural wall components.
(B) Both target viral RNA.
(C) One acts as a promoter for the other.
(D) They neutralize bacterial toxins.

2005

**187.** The P5CS enzyme catalyzes the rate-limiting step in the synthesis of:
(A) Proline
(B) Glycine βine
(C) Trehalose
(D) Sucrose

2016

**188.** Glyphosate acts by inhibiting the EPSPS enzyme, which is a crucial component of the shikimate pathway. This pathway is responsible for synthesizing:
(A) Aromatic amino acids
(B) Branched-chain amino acids
(C) Fatty acids
(D) Nucleic acids

2024

**189.** The gene encoding ACC deaminase, used to delay fruit ripening, was originally isolated from which soil bacterium?
(A) Pseudomonas chloroaphis
(B) Agrobacterium tumefaciens
(C) Bacillus subtilis
(D) Escherichia coli

2008

**190.** Match the trait in List I with the gene utilized to achieve it in List II.
List I: (Trait)
P. Golden Rice (Pro-Vitamin A)
Q. Herbicide resistance (Glufosinate)
R. Drought tolerance
S. Fungal resistance
List II: (Gene)
1. DREB1A
2. Chitinase
3. bar
4. psy
(A) P-IV, Q-III, R-I, S-II
(B) P-III, Q-IV, R-II, S-I
(C) P-IV, Q-I, R-III, S-II
(D) P-I, Q-III, R-IV, S-II

2013

**191.** High-laurate canola was developed by expressing a thioesterase gene from the California bay laurel. This enzyme acts by:
(A) Terminating elongation at the 12-carbon stage
(B) Desaturating oleic acid
(C) Elongating palmitic acid
(D) Breaking down triglycerides

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# ANSWERS

**1.** (A) Isolating a gene based on its genetic position and linkage to markers
Map-based cloning, also known as positional cloning, is a strategy used to identify and isolate a gene without prior knowledge of its function. It relies on high-resolution genetic mapping to find markers tightly linked to the target trait. By identifying the gene's physical location on a chromosome, researchers can narrow down and eventually clone the specific DNA sequence.

**2.** (C) It induces the expression of the Agrobacterium vir regulon.
Acetosyringone is a phenolic compound naturally released by wounded plant cells during the infection process. In Agrobacterium-mediated transformation, it acts as a critical signaling molecule that is sensed by the VirA protein. This interaction triggers a phosphorylation cascade that activates the transcription of virulence genes, which are essential for transferring T-DNA from the bacterium into the plant genome.

**3.** (A) It houses T-DNA and vir genes on two separate replicons.
The binary vector system is a more efficient approach for plant transformation compared to older co-integrative systems. It utilizes two distinct plasmids within the Agrobacterium cell to manage the transformation process. One plasmid carries the T-DNA containing the gene of interest, while a separate helper Ti plasmid provides the necessary virulence genes in trans to facilitate the DNA transfer.

**4.** (D) The helium gas pressure and target distance
Successful gene delivery through particle bombardment requires precise control over the physical parameters of the bombardment process. The pressure of the helium gas determines the velocity of the DNA-coated microcarriers, while the target distance affects how effectively they penetrate the plant tissue. Optimizing these two factors is essential for maximizing transformation efficiency while minimizing cellular damage to the explants.

NODIA

**5.** (B) Cauliflower Mosaic Virus (CaMV) 35S promoter
The Cauliflower Mosaic Virus 35S promoter is a powerful tool in plant genetic engineering due to its constitutive nature. It drives high levels of transgene expression across nearly all plant tissues and throughout various stages of development. Unlike tissue-specific or inducible promoters, it remains active regardless of environmental signals, providing a stable and reliable method for expressing desired transgenic traits.

(C) Kanamycin
The nptII gene, derived from the transposon Tn5, encodes the enzyme neomycin phosphotransferase II. This enzyme is widely used as a selectable marker in plant transformation because it confers resistance to several aminoglycoside antibiotics, most importantly kanamycin. By phosphorylating the antibiotic, the enzyme prevents it from binding to ribosomes, allowing only transformed plant cells to survive and grow on selection media.

**7.** (D) To isolate unknown genomic DNA flanking the transgene insertion site
Thermal Asymmetric Interlaced PCR is a specialized technique designed to amplify unknown DNA sequences adjacent to a known segment. In transgenic research, it is primarily used to identify the specific genomic location where a transgene has integrated. By using a series of nested specific primers and shorter degenerate primers, this method efficiently recovers the flanking genomic sequences for analysis.

**8.** (A) Southern blot hybridization
Southern blot hybridization remains the definitive molecular technique for verifying the stable integration of a transgene into the plant genome. This method involves digesting genomic DNA with restriction enzymes, separating the fragments by size, and using a labeled probe to detect the transgene. It provides crucial information regarding the presence, organization, and total copy number of the inserted DNA.

(A) It binds to unknown genomic sequences flanking the site.
(B) It binds to a specific known sequence within the transgene.
(C) It attaches to the poly-A tail of mRNA.
(D) It degrades unbound DNA.

2010

**204.** Quantitative Real-Time PCR (qRT-PCR) normalizes target gene expression against an internal control, typically:
(A) A viral gene
(B) A reference/housekeeping gene
(C) The selectable marker gene
(D) An intron sequence

2017

**205.** The primary reason Bt formulations are safe for human consumption is that:
(A) Humans degrade the toxin in the mouth.
(B) The acidic stomach destroys it; receptors are absent.
(C) Humans have antibodies against Bt.
(D) The toxin is only expressed in plant roots.

2016

**206.** Which of the following non-Bt insect resistance genes encodes an enzyme that disrupts the insect's ability to digest starch?
(A) A-amylase inhibitor
(B) Protease inhibitor
(C) Chitinase
(D) Lectin

2019

**207.** RNAi constructs designed for virus resistance typically contain:
(A) Inverted viral repeats designed to form a hairpin
(B) A full-length functional viral replicase gene
(C) A strong viral promoter driving a bacterial toxin
(D) The entire viral genome

2002

**208.** Which defense response is characterized by a rapid, localized cell death at the site of pathogen infection to restrict its spread?
(A) Systemic Acquired Resistance (SAR)
(B) The Hypersensitive Response (HR)
(C) Pathogen-Derived Resistance (PDR)
(D) RNA interference

2003

**209.** The accumulation of Late Embryogenesis Abundant (LEA) proteins is highly correlated with plant tolerance to:
(A) Desiccation and dehydration
(B) Herbicide application
(C) Viral infection
(D) Flooding

2004

**210.** Consider the following statements regarding glyphosate resistance:
I. Glyphosate targets the EPSPS enzyme.
II. The mutant CP4 EPSPS gene provides tolerance.
III. The GOX gene degrades glyphosate.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2010

**211.** Which fruit crop was the first to be genetically engineered for delayed ripening and approved for commercial sale (Flavr Savr)?
(A) Apple
(B) Tomato
(C) Banana
(D) Papaya

2010

**212.** To synthesize β-carotene (provitamin A) in rice endosperm, the Golden Rice 2 event successfully utilized the phytoene synthase (psy) gene from which plant?
(A) Daffodil
(B) Carrot
(C) Maize
(D) Tomato

2019

**213.** Silencing the FAD2 gene in crops like soybean and canola leads to a high-oleic acid phenotype. Why is high-oleic oil preferred industrially?
(A) It provides the plant with a natural insecticidal defense.
(B) It has a higher melting point, remaining solid at room temperature.
(C) It is more oxidatively stable and healthier for human consumption.
(D) It evaporates quickly during the commercial frying process.

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**18.** (C) It reduces gene flow risks via maternal inheritance of the transgene.
Plastid transformation offers several advantages over nuclear transformation, particularly regarding environmental safety. Because chloroplasts in most crop species are inherited maternally through the egg cell, the transgenes are not present in the pollen. This significantly reduces the risk of transgene escape to wild relatives or neighboring non-GM crops through cross-pollination, addressing a major concern in biosafety.

**19.** (A) Hypermethylation of the transgene promoter
Transcriptional gene silencing is a common phenomenon in transgenic plants where the expression of an inserted gene is suppressed at the level of mRNA synthesis. This event is most frequently linked to the epigenetic modification of DNA, specifically the hypermethylation of cytosine residues within the transgene promoter. This modification alters chromatin structure, making the DNA inaccessible to the transcriptional machinery.

**20.** (A) Both A and R are true and R is the correct explanation of A.
The Cre-loxP system is a powerful tool for genome engineering that enables the removal of unwanted DNA sequences, such as selectable markers. Cre is a site-specific recombinase that recognizes 34-base pair loxP sites. When a sequence is flanked by two loxP sites in the same orientation, the recombinase precisely excises the intervening DNA, leaving behind marker-free transgenic plants.

**21.** (B) Genetic Engineering Appraisal Committee (GEAC)
Under the Indian biosafety regulatory framework, the Genetic Engineering Appraisal Committee is the statutory body responsible for evaluating and approving the commercial release of genetically modified organisms. It functions under the Ministry of Environment, Forest and Climate Change. The committee assesses extensive data on environmental safety, human health impacts, and agronomic performance before granting permission for large-scale cultivation and use.

**22.** (C) Compositionally and nutritionally similar crops are safety-equivalent.
Substantial equivalence is a fundamental principle used in the safety assessment of genetically modified foods. It posits that if a GM crop is found to be compositionally and nutritionally similar to its conventional counterpart, it can be considered as safe as the traditional variety. This approach focuses on comparing key components like proteins, fats, vitamins, and minerals.

**23.** (D) Identifying genes differentially expressed between mRNA populations
Subtractive hybridization is a powerful technique used in molecular biology to identify genes that are uniquely expressed in one cell type or under specific conditions. By removing common sequences found in two different mRNA populations, researchers can enrich the specific transcripts that are upregulated or downregulated. This method is particularly useful for discovering genes involved in stress responses or development.

**24.** (A) VirD2
The VirD2 protein plays a crucial role in the processing of T-DNA during Agrobacterium-mediated transformation. It acts as a site-specific endonuclease that recognizes and nicks the double-stranded DNA at the conserved 25-base pair border sequences. After the nicking process, VirD2 remains covalently attached to the 5' end of the single-stranded T-DNA, guiding it into the host plant nucleus.

**25.** (B) It lacks tumor-forming oncogenes.
In plant biotechnology, a "disarmed" Ti plasmid is one that has been genetically modified to remove the oncogenes responsible for causing crown gall disease. These oncogenes, which typically encode enzymes for auxin and cytokinin synthesis, are replaced with the transgene of interest and selectable markers. This modification allows for the delivery of specific genes without inducing uncontrolled tumorous cell growth.

**26.** (B) Polyethylene glycol (PEG)
Polyethylene glycol is a chemical agent frequently used to facilitate the direct uptake of DNA into plant protoplasts. It works by altering the permeability of the plasma membrane and promoting the precipitation of DNA onto the cell surface. This method allows for the transformation of various plant species, including those that are resistant to Agrobacterium infection, by bypassing the cell wall.

NODIA

stranded DNA

2008

**226.** Which rapid, field-based immunodiagnostic tool is commonly used to detect the presence of Cry proteins in GM seeds or leaves?
(A) Northern blot
(B) Lateral flow strip (Dipstick)
(C) qRT-PCR
(D) Southern blot

2018

**227.** In the "high-dose/refuge" strategy, the "high dose" is defined as a concentration of Bt toxin that kills what percentage of susceptible insects?
(A) 50%
(B) 75%
(C) 95%
(D) 99%

2006

**228.** Which characteristic makes GNA (Galanthus nivalis agglutinin) lectin useful in plant biotechnology?
(A) It binds sap-sucking insect midgut glycoproteins.
(B) It is toxic to humans.
(C) It degrades viral DNA.
(D) It functions as a herbicide.

2022

**229.** RNAi-mediated virus resistance is highly specific. It will typically fail if the infecting virus:
(A) Has a DNA genome instead of RNA
(B) Carries a significant transgenic mismatch mutation
(C) Invades the roots instead of the leaves
(D) Is transmitted by an insect vector

2015

**230.** Consider the following statements concerning genetic engineering for disease resistance:
I. Overexpression of chitinase provides fungal resistance.
II. Phytoalexins are pre-formed structural barriers.
III. NPR1 overexpression enhances broad-spectrum disease resistance.
IV. Sarcotoxins are antibacterial peptides.
V. B-1,3-glucanase degrades bacterial cell walls.
Which of the above statement(s) is/are correct?
(A) I, III and IV
(B) II, III and V
(C) I, II and IV
(D) I, IV and V

2018

**231.** Proline acts as an osmoprotectant during drought stress. It protects the cell by:
(A) Maintaining balance and protein/membrane stability
(B) Hydrating the cell wall
(C) Closing the stomata physically
(D) Degrading reactive oxygen species directly as an enzyme

2002

**232.** The bar gene provides resistance against glufosinate by:
(A) Altering the target enzyme, glutamine synthetase
(B) Degrading glufosinate into a non-toxic compound
(C) Acetylating glufosinate to render it inactive
(D) Overproducing glutamine synthetase

2017

**233.** In fruit ripening engineering, ACC synthase and ACC oxidase are targeted because they are the key enzymes in the biosynthesis of:
(A) Auxin
(B) Cytokinin
(C) Ethylene
(D) Abscisic acid

2002

**234.** The integration of the ferritin gene aims to increase iron content. To ensure iron is stored predominantly in the edible seed endosperm, the ferritin gene is usually driven by a:
(A) Seed-specific promoter
(B) Constitutive promoter
(C) Root-specific promoter
(D) Light-inducible promoter

2022

**235.** Altering the lipid profile by silencing FAD2 prevents the conversion of:
(A) Stearic acid to oleic acid
(B) Oleic acid to linoleic acid
(C) Linoleic acid to linolenic acid
(D) Palmitic acid to stearic acid

2008

**236.** A significant advantage of chloroplast transformation over nuclear transformation is that chloroplasts:
(A) Do not require selectable markers
(B) Can correctly process and splice complex plant introns
(C) Allow for precise transgene integration via recombination

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2006

**192.** The state in which all copies of the plastid genome in a cell harbor the transgene is known as:
(A) Heteroplasmy
(B) Hemizygosity
(C) Homoplasmy
(D) Polyploidy

2011

**193.** The presence of inverted repeats of a transgene in the plant genome frequently leads to:
(A) Formation of dsRNA and subsequent gene silencing
(B) Exceptionally high levels of transgene expression
(C) Enhanced protein stability
(D) Autonomous replication of the transgene

2012

**194.** In a site-specific recombination system used for marker removal, heat-shock or chemical induction is often used to:
(A) Induce recombinase expression when required
(B) Kill the untransformed cells
(C) Activate the selectable marker
(D) Inactivate the T-DNA borders

2009

**195.** Before initiating any recombinant DNA experiments, a researcher in an Indian institute must obtain approval from the:
(A) RCGM
(B) District Magistrate
(C) IBSC
(D) GEAC

2014

**196.** The Tier-1 risk assessment for non-target organism effects of a Bt crop usually involves:
(A) Lab testing purified Bt protein on surrogate species
(B) Releasing the crop into a national park
(C) Spraying the crop with broad-spectrum insecticides
(D) Assessing human volunteers

2019

**197.** Map-based cloning relies heavily on:
(A) Genetic linkage mapping and chromosomal walking
(B) Knowing the protein sequence beforehand
(C) Random mutagenesis
(D) Homology to viral genomes

2005

**198.** The transfer of T-DNA from Agrobacterium to the plant cell is considered an evolutionary example of:
(A) Inter-kingdom horizontal gene transfer
(B) Vertical gene transfer
(C) Endosymbiosis
(D) Speciation

2015

**199.** Disarming a wild-type Ti plasmid entails the removal of the oncogenes. These oncogenes normally encode enzymes for the synthesis of:
(A) Auxin and cytokinin
(B) Opines
(C) Gibberellins
(D) Ethylene

NODIA

2015

**200.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
Assertion (A): Particle bombardment is particularly useful for transforming organelle genomes, such as plastids.
Reason (R): Gold or tungsten particles can physically penetrate the plant cell wall, plasma membrane, and organelle membranes to deliver DNA directly.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**201.** Which of the following is considered a tissue-specific promoter?
(A) Rubisco small subunit promoter
(B) Maize ubiquitin-1
(C) CaMV 35S
(D) Act1

2023

**202.** The mannose selection system (pmi) is deemed a "positive selection" system because:
(A) It kills non-transformed cells with a toxin.
(B) It confers a metabolic growth advantage on mannose.
(C) It positively charges the cell membrane.
(D) It results in a bright red phenotype.

2009

**203.** In TAIL-PCR, what is the function of the Arbitrary Degenerate (AD) primer?

**2002**

**248.** In Quantitative Real-Time PCR, a lower Ct (Cycle threshold) value indicates:
(A) A higher amount of target mRNA in the sample
(B) A lower amount of target mRNA in the sample
(C) Complete degradation of the RNA
(D) Inhibition of the PCR reaction

**2001**

**249.** Bt crops expressing Cry1Ac are highly effective against the cotton bollworm. This pest belongs to which insect order?
(A) Coleoptera
(B) Diptera
(C) Lepidoptera
(D) Orthoptera

**2010**

**250.** Which of the following best explains why "gene stacking" is preferred over single-gene resistance strategies?
(A) It requires simultaneous evolution of resistance mechanisms.
(B) It reduces the cost of producing transgenic seed.
(C) It eliminates the need for any biosafety regulations.
(D) It restricts cross-pollination with wild weeds.

**********

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(D) Only exist in monocots

2018

**237.** Which of the following is an effective strategy to mitigate Transcriptional Gene Silencing (TGS)?
(A) Flanking the transgene with Matrix Attachment Regions
(B) Utilizing highly repetitive promoter sequences
(C) Inserting inverted repeats of the transgene
(D) Culturing the plants at low temperatures

2010

**238.** In the FLP-FRT recombination system, the FLP recombinase catalyzes the excision of DNA located between two identically oriented FRT sites. This system requires:
(A) Crossing lines expressing FLP and carrying FRT sites
(B) Continuous application of a chemical inducer forever
(C) The presence of the Cre enzyme
(D) Homology to the Ti plasmid

2011

**239.** The primary role of an Institutional Biosafety Committee (IBSC) is to:
(A) Oversee institutional research within safety limits
(B) Market genetically modified crops
(C) Regulate the import of chemical fertilizers
(D) Conduct national-level clinical trials on humans

2007

**240.** Toxicity testing in GMO risk assessment typically involves feeding the purified transgenic protein to:
(A) Non-target insects
(B) Mice or rats for acute oral studies
(C) Human volunteers
(D) Farm animals like cows only

2022

**241.** Subtractive hybridization is utilized in gene isolation to:
(A) Isolate sequences common to two different DNA samples
(B) Enrich transcripts expressed in only one specific sample
(C) Sequence whole bacterial genomes
(D) Quantify the amount of a specific protein

NODIA

2001

**242.** Which Vir protein is required alongside VirD2 to recognize the T-DNA borders and possess topoisomerase activity?
(A) VirD1
(B) VirC1
(C) VirE1
(D) VirD4

2003

**243.** What structural feature defines the boundaries of the T-DNA on a Ti plasmid?
(A) The origin of replication
(B) 25-bp imperfect direct repeats at the ends
(C) The vir operon promoters
(D) The opine catabolism genes

2006

**244.** During particle bombardment, the microcarriers (gold or tungsten) are coated with DNA. Which chemical is typically used to precipitate the DNA onto the microcarriers?
(A) Calcium chloride and spermidine
(B) Polyethylene glycol (PEG)
(C) Acetosyringone
(D) Sodium dodecyl sulfate (SDS)

2019

**245.** Wound-inducible promoters are particularly useful in genetic engineering for:
(A) Localized insecticide expression at feeding sites
(B) Increasing nutritional content in seeds
(C) Enhancing drought tolerance in roots
(D) Modifying floral color

2009

**246.** The selectable marker pat, isolated from Streptomyces viridochromogenes, functions by:
(A) Adding an acetyl group to phosphinothricin
(B) Cleaving phosphinothricin
(C) Pumping phosphinothricin out of the cell
(D) Mutating the plant's glutamine synthetase

2017

**247.** To determine the exact copy number of a transgene using Southern blotting, the genomic DNA is digested with a restriction enzyme that:
(A) Cuts randomly millions of times within the transgene
(B) Cuts outside the T-DNA borders in the plant genome
(C) Cuts exactly once within the T-DNA sequence
(D) Does not cut the genomic DNA at all

**9.** (B) By binding to midgut receptors to form pores and cause cell lysis
Bacillus thuringiensis Cry proteins are highly specific toxins that target the digestive systems of certain insect pests. After ingestion, these crystalline protoxins are activated by midgut proteases and bind to specific receptors on the epithelial cell surface. This binding leads to the formation of ion-conducting pores, which causes osmotic swelling, cell rupture, and eventual death of the susceptible insect.

**10.** (A) P-IV, Q-I, R-II, S-III
Non-Bt insect resistance strategies utilize various biochemical mechanisms to disrupt pest physiology. Protease inhibitors interfere with protein digestion, while plant lectins bind to essential carbohydrates in the gut. A-amylase inhibitors block the breakdown of starch, starving the insect of energy. Additionally, vegetative insecticidal proteins (Vip) are secreted during bacterial growth and act as pore-forming toxins, similar to the Cry proteins.

**11.** (A) The transgenic plant delays or attenuates viral disease symptoms.
Pathogen-derived resistance utilizes segments of a virus's own genome to confer protection to the host plant. In coat protein-mediated resistance, the plant expresses the viral coat protein, which interferes with the early stages of the viral infection cycle, such as uncoating or replication. This typically results in a significant delay in symptom development or a reduction in disease severity.

**12.** (C) B-1,3-glucanase
B-1,3-glucanase is a specialized pathogenesis-related protein that plays a vital role in plant defense against fungal pathogens. It functions by enzymatically degrading β-glucans, which are major structural components of most fungal cell walls. By breaking down these polymers, the enzyme weakens the fungal structure and inhibits its growth, often working synergistically with other proteins like chitinases to enhance resistance.

**13.** (D) Proline
The P5CS gene encodes the enzyme delta-1-pyrroline-5-carboxylate synthetase, which catalyzes the rate-limiting step in the biosynthesis of proline. In plant biotechnology, overexpressing this gene leads to the increased accumulation of proline, which acts as a powerful osmoprotectant. This accumulation helps maintain cellular osmotic balance and protects proteins and membranes from damage during periods of drought or salinity stress.

**14.** (C) 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)
Glyphosate is a broad-spectrum herbicide that functions by inhibiting the EPSPS enzyme, a critical component of the shikimate pathway for synthesizing aromatic amino acids. To create glyphosate-resistant crops, researchers introduce a mutant version of the EPSPS gene, such as CP4 EPSPS. This modified enzyme possesses a much lower affinity for glyphosate, allowing the plant to maintain essential metabolic processes.

**15.** (B) Polygalacturonase (PG)
The Flavr Savr tomato was the first genetically engineered food crop to reach the market. Its delayed ripening phenotype was achieved by using antisense RNA technology to suppress the expression of the polygalacturonase enzyme. This enzyme is responsible for breaking down pectin in the cell walls, leading to fruit softening. Silencing this gene allowed the tomatoes to remain firm longer.

**16.** (D) The synthesis of β-carotene in the endosperm
Golden Rice was developed to address vitamin A deficiency by genetically engineering the carotenoid biosynthetic pathway in rice grains. By introducing the psy gene from daffodil and the crtI gene from a soil bacterium, researchers enabled the rice endosperm to produce β-carotene. This precursor is converted into vitamin A in the human body, providing a biofortified food source.

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**17.** (A) FAD2 and FAD3
Modifying the fatty acid composition of seed oils is a common goal in metabolic engineering. By silencing the FAD2 and FAD3 desaturase genes, researchers can reduce the levels of polyunsaturated fatty acids, such as linoleic and linolenic acids. This results in an increased proportion of oleic acid, which improves the oxidative stability and nutritional profile of the resulting vegetable oil.

2021

**214.** Plastid transformation is often achieved using the biolistic method. Which selectable marker is predominantly used to select for chloroplast transformants?
(A) nptII
(B) hpt
(C) bar
(D) aadA (confers spectinomycin resistance)

2008

**215.** Promoter methylation is a primary mechanism of Transcriptional Gene Silencing (TGS). Which nucleotide base is typically methylated?
(A) Adenine
(B) Guanine
(C) Cytosine
(D) Thymine

2009

**216.** In the co-transformation approach for generating marker-free plants, the selection of the marker-free progeny occurs in which generation?
(A) Callus phase
(B) T1 or subsequent segregating generations
(C) T0 generation
(D) Pre-transformation phase

2015

**217.** The Cartagena Protocol on Biosafety, an international agreement, primarily addresses:
(A) Safe handling, transport, and use of modified organisms
(B) The patenting of genetic sequences
(C) The banning of all chemical pesticides
(D) The promotion of organic farming

2021

**218.** In India, BRL-II (Biosafety Research Level II) field trials are permitted only after successful completion of:
(A) Commercialization
(B) Export of the seed
(C) Animal feeding studies on the wild type
(D) BRL-I trials

2006

**219.** Which method involves using the Agrobacterium T-DNA itself as a mutagen to identify and clone genes by their disruption?
(A) Map-based cloning
(B) Activation tagging
(C) Forward genetics
(D) Insertional mutagenesis via T-DNA

2015

**220.** Consider the following statements regarding the Agrobacterium vir regulon:
I. VirA acts as a receptor for plant phenolics.
II. VirG is phosphorylated and acts as a transcriptional activator.
III. VirD2 coats the single-stranded T-DNA.
IV. VirB proteins form the secretion channel.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) III only
(C) II and IV
(D) I, II and IV

2025

**221.** A binary vector system consists of:
(A) A Ti plasmid and an Ri plasmid
(B) T-DNA plasmid and separate helper vir plasmid
(C) Two identical plasmids in the same cell
(D) A plasmid and a plant chromosome

2008

**222.** Which technique utilizes a fine glass capillary to physically inject DNA directly into the plant nucleus?
(A) Biolistics
(B) Electroporation
(C) Microinjection
(D) PEG-mediated transfer

2009

**223.** The Rubisco small subunit promoter is regulated by:
(A) Wounding
(B) Temperature
(C) Light
(D) Drought

2011

**224.** The pmi gene is derived from E. coli. What enzyme does it encode?
(A) Phosphomannose isomerase
(B) Phosphinothricin acetyltransferase
(C) Neomycin phosphotransferase
(D) Hygromycin phosphotransferase

2019

**225.** A Northern blot specifically requires which type of probe to detect the target sequence?
(A) Labeled nucleic acid probe complementary to RNA
(B) An antibody specific to the RNA
(C) A protein kinase
(D) A fluorescent dye that binds all double-

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**27.** (A) Monocotyledonous plants
The maize ubiquitin-1 promoter is a highly effective constitutive promoter used to drive transgene expression in monocotyledonous crops like rice, wheat, and maize. It provides high levels of gene expression across most plant tissues and developmental stages. While many viral promoters like CaMV 35S are less active in monocots, the ubiquitin-1 promoter remains robust and reliable for cereal biotechnology.

**28.** (C) Mannose
The *pmi* gene encodes the enzyme phosphomannose isomerase, which is used in a positive selection system for plant transformation. Most plant cells cannot utilize mannose as a carbon source because they lack this enzyme. When the pmi gene is introduced, transformed cells gain the ability to convert mannose-6-phosphate into fructose-6-phosphate, allowing them to grow on media where mannose is the sole sugar.

**29.** (D) DNA
Southern blotting is a fundamental laboratory technique used to detect specific DNA sequences within a complex mixture. The process involves separating DNA fragments by gel electrophoresis, denaturing them into single strands, and transferring them onto a solid membrane. Once immobilized on the membrane, the DNA can be hybridized with a labeled probe to identify sequences that are complementary to the probe.

**30.** (C) I and II
Quantitative Real-Time PCR is a highly sensitive method used to measure the abundance of specific nucleic acid sequences. In transgene analysis, it is commonly used to quantify the level of mRNA transcripts produced by the inserted gene. The process requires an initial step where RNA is converted into cDNA. Unlike Western blotting, it does not directly measure the concentration of proteins.

**31.** (A) Bt toxin-expressing crops
The refuge strategy is a mandatory management practice designed to delay the development of insect resistance to Bt transgenic crops. It involves planting a specific percentage of non-Bt crops in or around the Bt fields. This provides a habitat for susceptible insects to thrive and mate with any rare resistant individuals, thereby diluting the frequency of resistance alleles in the population.

**32.** (C) Plant lectins
Plant lectins are a group of carbohydrate-binding proteins that serve as a natural defense mechanism against various insect pests. When ingested by insects, these lectins bind to specific glycoproteins in the peritrophic membrane or the midgut epithelium. This binding interferes with nutrient absorption and disrupts the integrity of the digestive tract, leading to reduced growth, impaired development, or mortality.

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**33.** (D) Triggering sequence-specific viral RNA degradation
RNA interference is a powerful natural mechanism used by plants to defend against viral infections. In transgenic plants, this technology is utilized to produce double-stranded RNA molecules that match the sequence of a target virus. These dsRNAs are processed into small interfering RNAs, which guide a protein complex to specifically recognize and degrade the viral RNA, effectively blocking viral replication.

**34.** (C) Antimicrobial compounds synthesized de novo after infection
Phytoalexins are low-molecular-weight antimicrobial compounds that plants produce in response to pathogen attack or environmental stress. Unlike pre-formed structural barriers, these secondary metabolites are synthesized de novo only when the plant perceives an infection. They exhibit broad-spectrum activity against fungi and bacteria, playing a critical role in the plant's localized defense response to restrict the spread of the pathogen.

**35.** (B) Cold and drought stress
The DREB1/CBF transcription factors are master regulators of the plant's response to abiotic stresses, particularly cold and drought. These proteins bind to the Dehydration-Responsive Element sequences in the promoters of numerous stress-inducible genes. Activation of this regulon leads to the accumulation of various protective molecules, such as osmolytes and chaperones, which enhance the plant's ability to survive harsh environmental conditions.

**36.** (D) bar or pat
Resistance to glufosinate, the active ingredient in herbicides like Basta and Liberty, is achieved by introducing the bar or pat genes. These genes, isolated from Streptomyces species, encode the enzyme phosphinothricin acetyltransferase. This

enzyme detoxifies the herbicide by adding an acetyl group to the phosphinothricin molecule, preventing it from inhibiting glutamine synthetase and allowing the transgenic plant to survive.

**37.** (A) Preventing the conversion of ACC to ethylene
Extending the shelf-life of fruits can be achieved by genetically engineering the ethylene biosynthesis pathway. ACC oxidase is the enzyme responsible for the final step of this pathway, converting 1-aminocyclopropane-1-carboxylic acid into ethylene gas. By downregulating the expression of this enzyme, researchers can significantly reduce ethylene production, which delays fruit ripening and softening, allowing for longer storage and transport.

**38.** (A) Fortifying the crop with iron
Ferritin is a ubiquitous protein responsible for the safe and efficient storage of iron within cells. In metabolic engineering, the introgression of a ferritin gene into staple crops is a strategy aimed at biofortification. By overexpressing ferritin in edible tissues like the grain endosperm, researchers can increase the total iron content, helping to address iron-deficiency anemia in populations reliant on these crops.

**39.** (A) California bay laurel
To produce rapeseed oil with high levels of laurate, a 12-carbon saturated fatty acid, scientists introduced a gene encoding a specialized thioesterase. This gene was isolated from the California bay laurel tree. The enzyme acts by specifically terminating the elongation of fatty acid chains at the 12-carbon stage, leading to the accumulation of lauric acid in the seeds of transgenic canola.

**40.** (B) Introduction of recombinase → Recombination at specific sites → Excision of the marker gene → Recovery of marker-free progeny
Generating marker-free transgenic plants using site-specific recombinases involves a precise series of steps. First, the recombinase gene is introduced into a plant containing a marker flanked by recognition sites. The recombinase then mediates a specific recombination event at these sites. This results in the physical excision of the marker gene, after which marker-free progeny are recovered in the next generation.

**41.** (C) Homologous recombination
Integration of transgenes into the plastid genome, such as the chloroplast, occurs almost exclusively through homologous recombination. This mechanism utilizes DNA sequences flanking the transgene that are identical to specific regions of the plastid genome. This allows for extremely precise and predictable gene insertion, avoiding the random integration patterns and associated "position effects" commonly observed during nuclear transformation in plants.

**42.** (C) Matrix Attachment Regions (MARs)
Matrix Attachment Regions are specific DNA sequences that bind to the nuclear scaffold, organizing chromatin into independent loops. Incorporating these elements into transformation vectors can help stabilize transgene expression and minimize post-transcriptional gene silencing. By shielding the transgene from the influence of surrounding chromatin and potential silencing signals, MARs promote more consistent and higher levels of gene expression in transgenic plants.

**43.** (D) Saccharomyces cerevisiae
The FLP-FRT excision system is a site-specific recombination tool derived from the 2-micron plasmid of the yeast Saccharomyces cerevisiae. It consists of the FLP recombinase protein and its corresponding 34-base pair recognition sites called FRT sites. Like the Cre-loxP system, it is used in plant biotechnology to precisely excise DNA sequences, such as selectable markers, to create cleaner transgenic products.

**44.** (D) Agronomic performance and environmental safety
Confined field trials are a mandatory step in the regulatory evaluation of genetically engineered crops. These trials are conducted under strictly controlled conditions to assess the agronomic performance of the crop in a real-world environment. More importantly, they allow researchers and regulators to monitor potential environmental safety issues, such as gene flow to wild relatives or impacts on non-target insect species.

**45.** (B) IBSC
The Institutional Biosafety Committee is the first level of regulatory oversight in the Indian biosafety framework. Every institution engaged in recombinant DNA research must establish an

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IBSC. Its primary responsibility is to monitor ongoing research activities, ensuring that they comply with safety guidelines and that potential risks to the environment and human health are properly managed within the laboratory and greenhouse.

**46.** (D) Inserting a known mobile element to tag a gene of interest
Transposon tagging is a powerful forward-genetics approach used to isolate and identify genes based on their function. It relies on the ability of transposable elements to move and insert themselves into various locations in the genome. When a known transposon disrupts a gene of interest, it creates a mutation and simultaneously "tags" the gene, allowing researchers to clone it easily.

**47.** (A) VirA and VirG
The Agrobacterium virulence system is regulated by a classic two-component regulatory system consisting of the VirA and VirG proteins. VirA is a membrane-bound sensor kinase that detects phenolic compounds and sugars released from wounded plant tissues. Upon sensing these signals, VirA autophosphorylates and subsequently transfers the phosphate group to VirG, which then acts as a transcriptional activator for other virulence genes.

**48.** (C) The vir genes in trans for T-DNA transfer
In a binary vector system, the helper plasmid plays a vital role by providing the necessary virulence genes. While the T-DNA containing the target gene is located on a smaller, easy-to-manipulate binary vector, the helper plasmid remains in the Agrobacterium cell. It expresses the proteins required for T-DNA processing and transport, effectively delivering the DNA from the binary vector in trans.

**49.** (A) Stigmas after pollination
The pollen-tube pathway is a method of direct gene transfer that bypasses the need for tissue culture. It involves applying a DNA solution directly to the stigmas of a flower shortly after pollination has occurred. The DNA is then carried by the growing pollen tube into the ovule, where it can integrate into the zygote, potentially resulting in transgenic seeds.

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**50.** (C) III only
Reporter genes are essential tools in plant biotechnology for verifying successful gene expression. The gusA gene produces a visible blue color in the presence of the X-Gluc substrate, while the luc gene generates light via the luciferase enzyme. However, the Green Fluorescent Protein (GFP) is unique because it is auto-fluorescent and does not require any exogenous substrate to emit light.

**51.** (A) Napin promoter
Tissue-specific promoters are used to restrict the expression of a transgene to a particular plant organ or developmental stage. The napin promoter, derived from Brassica napus, is a well-characterized seed-specific promoter. It is highly active during the period of seed storage protein accumulation, making it an ideal choice for metabolic engineering projects aimed at modifying the nutritional content of oilseeds.

**52.** (C) The specific translated protein
Western blotting is a common laboratory technique used to identify and quantify specific proteins within a complex mixture. The process involves separating proteins by size through gel electrophoresis and transferring them onto a membrane. The membrane is then incubated with specific antibodies that bind only to the target protein, providing clear evidence of whether the transgene mRNA was successfully translated.

**53.** (C) Southern blot
Southern blotting is the most analytically robust technique for determining the copy number of a transgene integrated into the plant genome. By digesting genomic DNA with restriction enzymes that cut once within the T-DNA and hybridizing the fragments with a transgene-specific probe, researchers can count the number of bands to estimate how many independent integration events have occurred in the plant.

**54.** (D) They can lead to a metabolic penalty.
While constitutive promoters ensure continuous transgene expression, they can sometimes cause a metabolic penalty in the transgenic plant. This occurs because the plant diverts significant energy and resources toward synthesizing the transgenic protein in every tissue, even where it is not needed. This unnecessary resource drain

response to different treatments, developmental stages, or environmental conditions.

**109.** (A) Acetosyringone
Acetosyringone is a small phenolic molecule that acts as a potent inducer of the Agrobacterium virulence genes. It is naturally secreted by plant cells in response to physical wounding. When Agrobacterium encounters acetosyringone, the molecule binds to the VirA sensor protein, initiating a signaling process that leads to the mobilization and transfer of T-DNA, making it a critical component for successful plant transformation.

**110.** (D) A is false but R is true.
While it was once thought that monocots were completely resistant to Agrobacterium-mediated transformation, this has been proven false. Many monocot species, including major cereals, can now be transformed using optimized protocols. However, the reason they are more challenging is that they often do not produce enough of the specific phenolic signals, like acetosyringone, that are necessary to induce the bacterial virulence system.

**111.** (A) Electroporation of protoplasts
Electroporation is a method of direct gene transfer that is highly dependent on the use of protoplasts, which are plant cells that have had their cell walls enzymatically removed. Without the rigid cell wall barrier, the electrical pulses can effectively create temporary pores in the plasma membrane, allowing DNA to enter the cell. While efficient, this method requires a robust system for regenerating whole plants from individual protoplasts.

**112.** (C) Phaseolus vulgaris (Common bean)
The phaseolin promoter is a well-known seed-specific promoter originally isolated from the common bean. It is responsible for driving the expression of phaseolin, the major storage protein in the bean seeds. In plant biotechnology, this promoter is frequently used to ensure that a transgene of interest is expressed only during the seed development stage, making it ideal for nutritional enhancement or molecular farming in seeds.

**113.** (D) Can metabolize unique carbon sources
Positive selection systems, such as the one using the pmi gene, differ from traditional antibiotic-based selection. Instead of using a toxic agent to kill untransformed cells, these systems provide transformed cells with a unique metabolic advantage. By gaining the ability to utilize a specific carbon source like mannose, the transformed cells can grow and thrive while the non-transformed cells remain metabolically inactive or grow very slowly.

**114.** (A) Residual Agrobacterium cells containing vectors
In the early stages of analysis following Agrobacterium-mediated transformation, researchers must be cautious of false-positive results in PCR tests. These can occur if residual Agrobacterium cells, which still contain the transformation vector, are present in the plant tissue samples. This can lead to the amplification of the transgene sequence from the bacterial DNA rather than the integrated DNA in the plant genome, necessitating further confirmatory tests.

**115.** (B) The exponential phase
Quantitative Real-Time PCR works by monitoring the accumulation of the PCR product in real-time as the reaction progresses. For accurate quantification, measurements are taken during the exponential phase of the reaction. During this phase, the amount of product doubles with each cycle, and the reaction is not yet limited by depleting reagents or accumulating inhibitors, allowing for a precise calculation of the initial template amount.

**116.** (B) Domain II
The Cry toxins produced by Bacillus thuringiensis consist of three distinct structural domains, each with a specific function. Domain II is primarily responsible for the toxin's specificity and binding. It contains several exposed loops that interact directly with specific protein or glycolipid receptors on the surface of the insect's midgut epithelial cells. This binding is a critical step that determines which insect species will be susceptible to the toxin.

**117.** (A) Vip are secreted during growth; Cry are crystalline at sporulation.
Vegetative insecticidal proteins and Cry proteins differ significantly in their production and physical state within the bacterium. Cry proteins are synthesized during the sporulation phase and form large, insoluble crystalline inclusions.

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**64.** (A) Bromoxynil
The bxn gene encodes a specialized nitrilase enzyme that provides resistance to the herbicide bromoxynil. This herbicide works by inhibiting photosynthesis in susceptible plants. The bxn enzyme detoxifies the chemical by hydrolyzing it into a non-toxic compound. Originally isolated from the bacterium Klebsiella pneumoniae, this gene has been successfully used to create various herbicide-tolerant transgenic crop varieties.

**65.** (B) Binding competitively to the EPSPS active site
Glyphosate-tolerant crops often utilize a mutant version of the EPSPS enzyme that has been modified at its active site. In susceptible plants, glyphosate acts as a competitive inhibitor, binding to the enzyme and blocking its normal substrate. The specific mutation in the resistant enzyme prevents glyphosate from binding effectively while still allowing the enzyme to carry out its essential role in amino acid synthesis.

**66.** (C) Degrading ACC, the precursor of ethylene
Overexpressing the enzyme ACC deaminase is a biotechnology strategy used to delay fruit ripening and extend shelf-life. This enzyme works by breaking down 1-aminocyclopropane-1-carboxylic acid, which is the immediate chemical precursor of ethylene gas in plants. By reducing the available pool of ACC, the plant produced significantly less ethylene, thereby slowing down the natural ripening and softening processes in the fruit.

**67.** (D) Sequester and increase internal iron content
The primary goal of expressing a ferritin gene in transgenic plants is to enhance their nutritional value through biofortification. Ferritin is a specialized protein that can sequester and store thousands of iron atoms in a non-toxic, bioavailable form. By increasing ferritin levels in edible plant parts, researchers can significantly boost the internal iron content, helping to combat widespread iron deficiency in human populations.

**68.** (A) Oleic acid
Silencing the FAD2 gene is a common technique used to modify the lipid profile of oilseed crops. The FAD2 enzyme is responsible for converting oleic acid into linoleic acid by adding a double bond. When this gene is suppressed, the conversion process is blocked, leading to a significant accumulation of oleic acid in the seeds. High-oleic oils are more stable and healthier.

**69.** (C) Polycistronic transcription
A unique and highly advantageous feature of the chloroplast genome is its ability to perform polycistronic transcription. Like bacteria, chloroplasts can organize multiple genes into a single operon under the control of a single promoter. This allows researchers to introduce and express entire metabolic pathways or multiple resistance genes simultaneously, making plastid transformation a powerful tool for complex genetic engineering projects.

**70.** (A) P-III, Q-IV, R-II, S-I
Transgenic gene constructs consist of several essential elements, each with a specific function. The Rubisco small subunit promoter ensures expression primarily in green tissues. Selectable markers like the nptII gene provide resistance to antibiotics like kanamycin. Reporter genes like gusA allow for the visual confirmation of successful gene expression, while Matrix Attachment Regions (MARs) help stabilize expression by minimizing silencing.

**71.** (B) Sequence-specific degradation of homologous mRNA
Post-transcriptional gene silencing is a common regulatory mechanism in plants where the expression of a transgene is suppressed after the mRNA has been synthesized. This process involves the production of small interfering RNAs that match the sequence of the target mRNA. These siRNAs guide a protein complex to recognize and cleave the mRNA, preventing it from being translated into a protein.

**72.** (D) Generate marker-free plants via gene separation
Co-transformation is a strategy used to generate marker-free transgenic plants. It involves introducing the selectable marker and the gene of interest on two separate DNA fragments. If these two fragments integrate at different, unlinked locations in the plant genome, they will segregate independently during meiosis. In the next generation, researchers can select progeny that have inherited the gene of interest but not the marker.

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can result in reduced growth rates, lower yields, or other undesirable phenotypic changes.

**55.** (B) Cold-responsive promoter
Inducible promoters are DNA sequences that activate gene expression only in response to specific external stimuli. A cold-responsive promoter is a classic example of an abiotic stress-inducible promoter. It remains relatively inactive under normal growing conditions but becomes highly active when the plant is exposed to low temperatures, ensuring that protective transgenic products are produced only when they are needed.

**56.** (C) It acetylates phosphinothricin to detoxify it.
The bar gene encodes the enzyme phosphinothricin acetyltransferase, which provides resistance to the herbicide glufosinate. The enzyme functions by adding an acetyl group to the phosphinothricin molecule, which is the active component of the herbicide. This chemical modification renders the herbicide non-toxic, preventing it from inhibiting the plant's glutamine synthetase enzyme and allowing the transgenic plant to survive treatment.

**57.** (D) It is a single-stranded DNA binding protein for T-strand protection.
VirE2 is a crucial protein in the Agrobacterium-mediated transformation process. It functions as a single-stranded DNA binding protein that coats the T-strand once it is processed from the Ti plasmid. By binding along the length of the T-strand, VirE2 protects the DNA from degradation by plant nucleases and helps maintain its linear structure, facilitating its transport into the plant nucleus.

**58.** (A) Using multiple distinct Bt genes to delay resistance
Gene stacking, or pyramiding, is a strategy used in transgenic crop development to enhance the durability of pest resistance. It involves introducing two or more different Bt genes, each encoding a toxin with a distinct mode of action, into the same plant. This approach makes it much harder for insect populations to evolve resistance, as they would need to develop multiple mutations simultaneously.

**59.** (B) A modified viral replicase gene in the host
Replicase-mediated resistance is a form of pathogen-derived resistance where a transgenic plant expresses a version of the virus's own replicase gene. Often, a truncated or mutated form of the replicase is used. The presence of this transgenic protein interferes with the assembly or function of the natural viral replication complex, effectively inhibiting the virus's ability to multiply within the plant cells.

**60.** (A) 2, 1, 4, 3
RNA interference-mediated virus resistance follows a specific sequence of molecular events. First, a hairpin RNA construct is transcribed in the plant cell. This double-stranded RNA is recognized and cleaved by the Dicer enzyme into small interfering RNAs. These siRNAs are then incorporated into the RISC complex, which guides the degradation of matching viral RNA, successfully blocking the viral infection.

**61.** (D) By blocking the insect's ability to digest starch
A-amylase inhibitors are specialized proteins that contribute to plant defense by targeting the digestive enzymes of insect pests. When an insect ingests these inhibitors, they bind to and neutralize α-amylase in the midgut. This enzyme is essential for the breakdown of complex starches into simple sugars. By blocking starch digestion, the inhibitors starve the insect of its primary energy source.

**62.** (C) NPR1
NPR1 is a master regulatory protein that plays a central role in the systemic acquired resistance pathway in plants. Upon activation by salicylic acid, NPR1 moves into the nucleus and interacts with transcription factors to induce the expression of numerous pathogenesis-related genes. Manipulating NPR1 expression is a key strategy for developing transgenic crops with broad-spectrum and durable resistance to various pathogens.

**63.** (D) Glycine βine
Choline monooxygenase is a critical enzyme in the biosynthetic pathway of glycine βine, a highly effective osmoprotectant found in many plants. It catalyzes the first step of the pathway, converting choline into βine aldehyde. Overexpressing this enzyme in transgenic crops helps them accumulate glycine βine, which protects cellular structures and maintains osmotic balance during exposure to drought or salinity stress.

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**82.** (B) It requires specialized equipment and skilled staff.
While microinjection is a precise and effective method for delivering DNA directly into a cell's nucleus, it has significant practical drawbacks. The technique requires highly specialized and expensive equipment, such as micromanipulators and microinjectors. Additionally, it is a very labor-intensive process that demands a high level of skill and experience, making it difficult to use for large-scale transformation projects in plant research.

**83.** (C) Phosphinothricin
The pat gene is a widely used selectable marker that confers tolerance to phosphinothricin, the active ingredient in glufosinate herbicides. It encodes the enzyme phosphinothricin acetyltransferase, which detoxifies the herbicide by adding an acetyl group to the molecule. This prevents the herbicide from inhibiting the plant's glutamine synthetase, allowing only the transformed cells to survive and grow during the selection process.

**84.** (B) It is visualized non-destructively under UV light.
The Green Fluorescent Protein reporter system is highly valued in plant biotechnology because it allows for the non-destructive visualization of gene expression. When exposed to blue or ultraviolet light, the protein emits a bright green fluorescence that can be observed in living tissues. Unlike other reporter systems, GFP does not require any exogenous substrates or the destruction of the plant cells.

**85.** (B) Northern blotting detects RNA; Western blotting detects proteins.
Northern and Western blotting are fundamental techniques used to analyze gene expression at different molecular levels. Northern blotting is used to detect and quantify specific RNA molecules, providing information about gene transcription. In contrast, Western blotting is used to detect specific proteins, confirming that the mRNA has been successfully translated. Both techniques rely on separating molecules by size and using specific probes.

**86.** (C) An enzyme producing a colored product
In a standard ELISA, an enzyme is typically conjugated to the secondary antibody to allow for the detection of the target protein. When a substrate is added, the enzyme catalyzes a chemical reaction that produces a measurable signal, usually a color change. The intensity of the color is directly proportional to the amount of transgenic protein present in the sample, allowing for quantification.

**87.** (B) Inactive protoxins
Bacillus thuringiensis Cry proteins are initially synthesized as inactive protoxins that aggregate into crystalline inclusions during the bacterium's sporulation phase. These protoxins are not harmful to the bacterium itself or most other organisms. They only become toxic once they are ingested by a susceptible insect, where the alkaline conditions and specific proteases in the midgut cleave them into smaller, active toxin molecules.

**88.** (A) Lepidoptera
The Cry1 class of Bt toxins is highly specific and is primarily effective against insects in the order Lepidoptera, which includes many significant agricultural pests like bollworms and stem borers. Each class of Cry proteins has a narrow host range determined by the specific receptors present in the insect midgut, ensuring that the toxins are effective against target pests while remaining safe.

**89.** (B) Affect midgut cells without specific receptors
Cyt proteins are a different class of toxins produced by some Bacillus thuringiensis strains. Unlike Cry proteins, which require specific receptors to bind to midgut cells, Cyt proteins are thought to interact directly with the lipid components of the cell membrane. This allows them to exert their toxicity through a more general mechanism of membrane disruption, although they often show lower overall toxicity.

**90.** (A) I, II, III and V
The refuge strategy is a critical component of insect resistance management for Bt crops. It involves planting non-Bt crops to maintain a population of susceptible insects that can mate with any rare resistant individuals. This process keeps the frequency of resistance alleles low in the population. The use of multiple Bt genes, while helpful, is part of a gene stacking strategy.

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**73.** (B) Zygosaccharomyces rouxii
The site-specific recombinase "R" is part of a recombination system originally isolated from the yeast species Zygosaccharomyces rouxii. This system utilizes the R recombinase to recognize and act upon specific recognition sequences called RS sites. Like other recombinase systems, it is used in plant biotechnology to precisely manipulate DNA, including the removal of selectable markers from the genomes of transgenic plants.

**74.** (A) Ministry of Science and Technology
In India, the Review Committee on Genetic Manipulation is a key regulatory body that functions under the Department of Biotechnology within the Ministry of Science and Technology. The RCGM is responsible for overseeing and monitoring research activities involving genetically modified organisms, including greenhouse and confined field trials, to ensure that they are conducted safely and in compliance with national guidelines.

**75.** (D) Herbicide tolerance is transferred to wild weeds.
A major concern in the environmental risk assessment of genetically modified crops is the potential for gene flow. This occurs when a transgene, such as one conferring herbicide tolerance, is transferred to wild relatives or weeds through cross-pollination. If these weeds acquire the trait, they may become much harder to control, leading to the creation of "superweeds" that can thrive in agricultural landscapes.

**76.** (A) Amino acid sequence homology to known allergens
Assessing the potential allergenicity of a newly introduced transgenic protein is a critical step in food safety evaluations. One primary method involves comparing the amino acid sequence of the transgenic protein with a database of known human allergens. If significant sequence homology is found, it indicates a higher risk that the protein could trigger an allergic reaction in sensitive individuals.

**77.** (D) Restriction endonucleases
The construction of a genomic library begins with the fragmentation of the entire genome into smaller, manageable pieces of DNA. This is most commonly achieved using restriction endonucleases, which are enzymes that cut DNA at specific recognition sequences. These fragments are then inserted into suitable vectors and cloned, creating a collection that ideally represents the complete genetic information of the source organism.

**78.** (A) Reverse transcriptase
Reverse transcriptase is an essential enzyme used in the construction of cDNA libraries. It allows researchers to synthesize a complementary DNA strand using an mRNA molecule as a template. This step is crucial because it converts unstable, single-stranded RNA into more stable, double-stranded DNA, which can then be cloned and studied to identify the genes that were actively expressed in a specific tissue.

**79.** (D) The T-DNA region
The T-DNA region of the Agrobacterium Ti plasmid contains several genes that are transferred and integrated into the plant genome. Among these are genes responsible for the synthesis of opines, which are unusual amino acid or sugar derivatives. Once the plant cells are transformed, they begin to produce opines, which the Agrobacterium can then use as a specialized source of carbon and nitrogen.

**80.** (A) Both A and R are true and R is the correct explanation of A.
Binary vector systems are generally preferred over co-integrative systems for plant transformation due to their ease of use. Because the T-DNA and the virulence genes are located on two separate plasmids, the vectors are smaller and much easier to manipulate in common laboratory bacteria like E. coli. Furthermore, they do not require complex homologous recombination events within the Agrobacterium cell.

**81.** (C) Creating transient membrane pores via electricity
Electroporation is a physical method of direct gene transfer used to introduce DNA into plant cells, typically protoplasts. It works by applying short, high-voltage electrical pulses to a cell suspension. These pulses create temporary, nanometer-sized pores in the plasma membrane, allowing DNA molecules to enter the cytoplasm from the surrounding medium. After the pulse, the pores reseal, leaving the cells transformed.

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**100.** (A) P-IV, Q-I, R-III, S-II
Selectable marker genes are paired with specific selecting agents to identify transformed plant cells. The hpt gene provides resistance to hygromycin, while the bar gene confers tolerance to phosphinothricin. The nptII gene allows for selection on kanamycin, and the pmi gene enables cells to utilize mannose as a carbon source. These systems ensure that only cells containing the transgene can survive and regenerate.

**101.** (A) Reducing polyunsaturates by silencing desaturases
Improving the stability and shelf-life of frying oils can be achieved by reducing the concentration of polyunsaturated fatty acids, which are highly susceptible to oxidation. By silencing the desaturase enzymes responsible for adding double bonds to fatty acids, researchers can create transgenic oilseed varieties with higher levels of monounsaturated oleic acid. This modification results in oils that are more resistant to heat-induced breakdown.

**102.** (C) Sequences homologous to the chloroplast genome
In plastid transformation, precise targeting of the transgene is achieved through the mechanism of homologous recombination. To ensure the transgene integrates at a specific, desired location, it must be flanked by DNA sequences that are identical to the target site in the chloroplast genome. These flanking sequences guide the transgene into the genome, allowing for stable and predictable expression without the risks of random integration.

**103.** (C) Plastid transformation
One of the unique advantages of plastid transformation is its ability to express multiple transgenes as a single multi-gene operon. This polycistronic expression, which is characteristic of the prokaryotic-like nature of the chloroplast genome, allows researchers to introduce entire metabolic pathways or multiple traits simultaneously under the control of a single promoter. This is far more efficient than trying to coordinate multiple independent genes in the nucleus.

**104.** (B) Anchoring chromatin to shield from silencing
Matrix Attachment Regions are DNA sequences that bind to the nuclear matrix, organizing the genome into structural and functional loops. When incorporated into transgene constructs, MARs help to stabilize gene expression by providing a physical shield against the silencing effects of surrounding heterochromatin. This helps to reduce "position effects" and ensures more consistent and reliable expression of the transgene across different transformants.

**105.** (C) Excision of the marker gene
In the site-specific Cre-loxP recombination system, the orientation of the loxP sites determines the outcome of the recombinase action. If a DNA sequence, such as a selectable marker, is flanked by two loxP sites oriented in the same direction, the Cre recombinase will precisely excise the intervening DNA. This mechanism is frequently used to remove unwanted genes from the genome of transgenic plants, creating cleaner final products.

**106.** (B) DBT
To ensure strict compliance with national safety guidelines, every Institutional Biosafety Committee must include a nominee from the Department of Biotechnology. This nominee provides an external layer of oversight and ensures that the institution's research involving genetically modified organisms is conducted in accordance with the legal and safety frameworks established by the Indian government. This helps maintain high standards of biosafety across the country.

**107.** (B) Phenotypic traits and potential for weediness
During Biosafety Research Level I field trials, researchers evaluate various parameters to assess the safety and performance of a genetically modified crop. A key focus is on characterizing the crop's phenotypic traits and monitoring its potential for weediness or invasiveness in the environment. These trials are conducted under confined conditions to collect essential data before moving to larger-scale field evaluations.

**108.** (B) Identify differentially expressed mRNAs
Differential display is a transcriptomics technique used to compare the patterns of gene expression in two or more different biological samples. By using a combination of anchored primers and short arbitrary primers, researchers can amplify and visualize subsets of the mRNA population. This allows for the identification of specific genes that are upregulated or downregulated in

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**91.** (B) They bind to different receptors than Cry proteins.
Vegetative insecticidal proteins (Vip) are a valuable tool in pest management because they possess a different mode of action than the more common Cry proteins. Because Vip proteins bind to different receptors in the insect midgut, there is little to no cross-resistance between the two. Using both types of proteins together in a single plant significantly delays the evolution of insect resistance.

**92.** (B) Sap-sucking insects (e.g., aphids)
The GNA lectin, isolated from the snowdrop plant, is particularly effective against sap-sucking insects in the order Hemiptera, such as aphids and planthoppers. When these insects feed on transgenic plants expressing the lectin, the protein binds to glycoproteins in their digestive tract. This binding disrupts normal nutrient absorption and physiological processes, leading to reduced survival and reproductive rates in the pest populations.

**93.** (A) It is often narrowly specific to the source virus.
One major limitation of pathogen-derived resistance, particularly when using viral coat proteins, is its high level of specificity. The resistance conferred is typically restricted to the specific virus from which the gene was derived or very closely related strains. If the plant is attacked by a different or highly mutated virus, the transgenic coat protein may not provide any significant protection.

**94.** (D) Insects
Cecropins are a group of potent antimicrobial peptides that were originally discovered in the pupae of the giant silk moth. These peptides are a key component of the insect's innate immune system, helping it fight off bacterial infections. In plant biotechnology, genes encoding cecropins have been used to develop transgenic varieties with enhanced resistance to a wide range of bacterial and fungal pathogens.

**95.** (A) Pathogenesis-Related (PR) genes
NPR1 is a central hub in the signaling pathway for systemic acquired resistance in plants. When a plant perceives a pathogen attack, the accumulation of salicylic acid leads to the activation and nuclear translocation of NPR1. Once in the nucleus, it acts as a transcriptional co-activator, inducing the expression of numerous pathogenesis-related genes that provide the plant with broad-spectrum and long-lasting immunity.

**96.** (A) Acting as chaperones to prevent denaturation
Late Embryogenesis Abundant proteins are thought to play a vital role in protecting plant cells during severe dehydration or drought. These highly hydrophilic proteins are believed to function as molecular chaperones, binding to other proteins and membranes to prevent them from denaturing or aggregating as the cell loses water. By maintaining cellular integrity, LEA proteins help plants survive extreme desiccation stress.

**97.** (B) Degrading glyphosate into harmless products
The introduction of the glyphosate oxidoreductase gene is a strategy used to confer herbicide tolerance in transgenic crops. Unlike other methods that modify the target enzyme, the GOX enzyme works by directly breaking down the glyphosate molecule. It converts glyphosate into glyoxylate and aminomethylphosphonic acid, both of which are non-toxic to the plant, effectively neutralizing the herbicide before it can cause damage.

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**98.** (C) Complementary to the target endogenous mRNA
Antisense RNA technology involves introducing a gene construct that produces an RNA strand complementary to a specific target mRNA in the plant. When these two strands meet, they hybridize to form a double-stranded RNA molecule. This prevents the target mRNA from being translated by ribosomes and often triggers its degradation, effectively silencing the gene and allowing researchers to study its function or modify traits.

**99.** (B) Fungal phytase expression to break phytic acid
Phytic acid is a major anti-nutritional factor in many feed crops, as it binds to essential minerals and makes them unavailable for absorption by monogastric animals. A metabolic engineering approach to address this involves expressing a fungal phytase enzyme in the transgenic plant. This enzyme breaks down phytic acid into inorganic phosphorus and inositol, improving the nutritional quality of the feed and reducing environmental pollution.

In contrast, Vip proteins are produced and secreted into the surrounding medium during the vegetative growth phase of Bacillus thuringiensis. This difference allows for their recovery from the culture supernatant rather than from the cell pellet.

**118.** (D) Host-Induced Gene Silencing (HIGS) via RNAi
Host-induced gene silencing is a revolutionary transgenic approach used to control insect pests and pathogens. It involves engineering the host plant to produce double-stranded RNA molecules that match the sequence of an essential gene in the target pest. When the insect feeds on the plant, it ingests these dsRNAs, which then trigger the RNA interference pathway within the insect's own cells, leading to gene silencing and mortality.

**119.** (B) The flesh fly (Sarcophaga peregrina)
Sarcotoxins are a class of antimicrobial peptides that were originally isolated from the larvae of the flesh fly. These peptides are produced as part of the insect's immune response to bacterial infection. In plant biotechnology, sarcotoxin genes have been introduced into various crop species to enhance their innate defense mechanisms, providing increased resistance against a broad range of bacterial pathogens that cause significant agricultural losses.

**120.** (B) II only
Transcription factors like DREB1/CBF are essential for regulating stress response networks in plants. Proline also serves as a critical osmoprotectant during drought stress. However, Late Embryogenesis Abundant (LEA) proteins are generally highly hydrophilic, not hydrophobic. Their high solubility and hydrophilic nature allow them to function as molecular shields or chaperones, protecting other cellular components from damage during severe dehydration or desiccation.

**121.** (A) Klebsiella pneumoniae ozaenae
The bxn gene, which confers resistance to the herbicide bromoxynil, was originally isolated from the soil bacterium Klebsiella pneumoniae subspecies ozaenae. This gene encodes a specialized nitrilase enzyme that can rapidly detoxify the herbicide. By integrating this gene into plant genomes, researchers have successfully developed various herbicide-tolerant crop varieties, allowing for more effective weed management in agricultural systems using bromoxynil-based products.

**122.** (C) SAM to 1-aminocyclopropane-1-carboxylic acid
ACC synthase is a key regulatory enzyme in the biosynthetic pathway of ethylene, a gaseous plant hormone that controls fruit ripening. It catalyzes the conversion of S-adenosylmethionine into 1-aminocyclopropane-1-carboxylic acid (ACC). Because this is the rate-limiting step in ethylene production, it is a primary target for genetic manipulation. Suppressing this enzyme can significantly reduce ethylene levels, thereby extending the shelf-life and firmness of various fruits.

**123.** (D) Phytoene desaturase
In the development of Golden Rice, the crtI gene plays a crucial role in completing the carotenoid biosynthetic pathway in the rice endosperm. This gene, isolated from the bacterium Erwinia uredovora, encodes the enzyme phytoene desaturase. This bacterial enzyme is particularly useful because it can perform multiple desaturation steps that normally require several different enzymes in plants, streamlining the process of converting phytoene into lycopene.

**124.** (C) The number of double bonds in the carbon chain
Desaturase enzymes are responsible for the formation of double bonds in the carbon chains of fatty acids, a process that determines the degree of saturation in plant oils. By targeting and modifying the activity of these enzymes through genetic engineering, researchers can control the ratio of saturated to unsaturated fats. This allow for the creation of oils with specific nutritional profiles or improved oxidative stability for industrial use.

**125.** (C) Gene silencing
A significant advantage of chloroplast transformation is its relative immunity to the gene silencing phenomena that often plague nuclear transgenes. In the nucleus, multiple copies of a gene can trigger transcriptional or post-transcriptional silencing mechanisms. However, the plastid genome appears to lack these specific silencing pathways, allowing for the stable and high-level expression of transgenes even when they are present in thousands of copies per cell.

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**143.** (D) Salinity or drought osmotic stress
The overexpression of the choline monooxygenase gene is a strategy used to enhance the tolerance of transgenic plants to osmotic stresses like salinity and drought. This enzyme catalyzes the first step in the biosynthesis of glycine βine, which is a highly effective osmoprotectant. By increasing the accumulation of glycine βine, the plant can better maintain cellular turgor and protect essential proteins and membranes from stress-induced damage.

**144.** (C) Phosphinothricin acetyltransferase (PAT)
The detoxification of the herbicide glufosinate in resistant transgenic plants is achieved by the enzyme phosphinothricin acetyltransferase. This enzyme is encoded by the bar or pat genes. It functions by adding an acetyl group to the glufosinate molecule, which is the active component of the herbicide. This modification inactivates the herbicide, preventing it from inhibiting the plant's glutamine synthetase enzyme and allowing the transgenic crop to survive.

**145.** (B) Cantaloupe melon
The use of antisense technology to target the ACC synthase gene was first successfully demonstrated as a method for delaying fruit ripening in cantaloupe melons. ACC synthase is a critical regulatory enzyme in the ethylene biosynthesis pathway. By suppressing its expression, researchers were able to significantly reduce ethylene production in the melons, leading to a delay in fruit softening and an extension of the fruit's overall shelf-life.

**146.** (A) The carotenoid biosynthetic pathway
The development of Golden Rice involved the significant genetic modification of the carotenoid biosynthetic pathway in the rice grain. By introducing external genes, researchers enabled the rice endosperm, which normally does not produce carotenoids, to synthesize β-carotene. This specific pathway was targeted because β-carotene is the primary precursor for vitamin A, making it a critical nutrient for addressing widespread vitamin A deficiency in many developing regions.

**147.** (B) Desaturases
Modifying the degree of saturation in plant oils is primarily achieved by altering the activity of fatty acid desaturases. These enzymes are responsible for introducing double bonds at specific positions along the carbon chains of fatty acids. By increasing or decreasing the expression of various desaturase genes, researchers can control the ratio of saturated, monounsaturated, and polyunsaturated fats in the seed oil, tailored to specific health or industrial needs.

**148.** (A) It addresses regulatory safety concerns.
Developing marker-free transgenic plants is a highly desirable goal in plant biotechnology because it addresses several significant regulatory and public safety concerns. There is concern that antibiotic or herbicide resistance genes could potentially spread to other organisms in the environment. By removing these markers after they have served their purpose in the selection process, researchers can create a cleaner and more acceptable transgenic product for commercial use.

**149.** (D) Cytosine methylation of the promoter
Transcriptional gene silencing is an epigenetic process that prevents the synthesis of mRNA from a transgene. A classic molecular hallmark of this phenomenon is the hypermethylation of cytosine residues within the DNA of the transgene promoter. This modification often leads to changes in chromatin structure, such as condensation into heterochromatin, which makes the DNA inaccessible to RNA polymerase and other essential transcriptional machinery.

**150.** (A) 2, 3, 1
The approval process for a new genetically modified crop in India follows a rigorous, step-by-step regulatory pathway. First, the research is evaluated at the institutional level by the IBSC. If approved, the data is reviewed by the RCGM to grant permission for confined field trials. Finally, after successful trials and a comprehensive safety assessment, the GEAC provides the ultimate approval for the large-scale commercial release of the crop.

**151.** (A) Evaluating protein resemblance to allergens
One of the primary methods used to assess the potential allergenicity of a newly introduced transgenic protein is to evaluate its sequence resemblance to known human allergens. Scientists use bioinformatics tools to compare the amino acid sequence of the transgenic protein with extensive databases of established allergens. Significant sequence similarity or the presence of

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electrical parameters. The strength of the electric field and the duration of the pulse must be precisely controlled to create enough transient pores for DNA entry without causing excessive cell death. Finding the right balance is essential for maximizing the number of transformed cells that remain viable and capable of regenerating into whole plants.

**135.** (B) Its activity is often lower in monocots.
The CaMV 35S promoter is a very strong and widely used constitutive promoter in dicotyledonous plants. However, in many monocot species, including major cereal crops, its overall activity is often significantly lower and less consistent. For this reason, researchers working with monocots often prefer to use promoters derived from monocot genes, such as the maize ubiquitin or rice actin promoters, to ensure high-level transgene expression.

**136.** (A) Phosphorylation
The hpt gene, which is commonly used as a selectable marker in plant transformation, encodes the enzyme hygromycin B phosphotransferase. This enzyme provides resistance to the antibiotic hygromycin B by catalyzing the phosphorylation of the antibiotic molecule. This chemical modification renders hygromycin B inactive, preventing it from inhibiting protein synthesis in the plant cells and allowing the transformed cells to survive on selection media.

**137.** (C) TAIL-PCR
Thermal Asymmetric Interlaced PCR is a powerful molecular tool used to recover unknown DNA sequences that are adjacent to a known segment. The technique involves three successive rounds of PCR using nested specific primers and a shorter arbitrary degenerate primer. By varying the annealing temperatures, the method selectively amplifies the flanking genomic DNA, which is particularly useful for identifying transgene insertion sites or cloning gene promoters.

**138.** (C) To block non-specific binding sites on the membrane
In the Western blot procedure, a blocking step is essential to ensure the specificity of the antibody binding. After the proteins have been transferred to a membrane, researchers incubate the membrane with a solution of non-fat dry milk or bovine serum albumin. These proteins bind to any empty spaces on the membrane surface, preventing the detection antibodies from binding non-specifically and reducing the background signal in the final assay.

**139.** (B) It requires simultaneous developer mutations.
Gene pyramiding is an effective resistance management strategy because it places an enormous evolutionary pressure on the target insect population. For an insect to survive on a pyramided crop, it would need to possess or develop multiple independent mutations that provide resistance to each of the different toxins simultaneously. The probability of such rare events occurring at the same time is extremely low, making the resistance much more durable.

**140.** (A) II only
Non-Bt insect resistance mechanisms target various aspects of pest physiology. Protease inhibitors interfere with protein breakdown, while plant lectins bind to essential glycoproteins in the gut. Vegetative insecticidal proteins (Vip) are unique, secreted toxins with different modes of action than Cry proteins. However, α-amylase inhibitors specifically target carbohydrate-digesting enzymes, not lipid-digesting pests, by blocking the breakdown of starch into sugars.

**141.** (A) The specific source virus and related strains
Coat protein-mediated resistance is a form of pathogen-derived resistance that provides transgenic plants with protection against viral infections. However, the spectrum of resistance is typically quite narrow. The protection is most effective against the specific virus from which the coat protein gene was originally isolated or very closely related strains. It generally does not provide broad-spectrum resistance to unrelated viruses or other types of pathogens.

**142.** (C) Fungi
Chitinases are a group of enzymes that are widely used in plant biotechnology to enhance resistance against fungal pathogens. They work by enzymatically breaking down chitin, a structural polysaccharide that is a primary component of fungal cell walls. By degrading this essential barrier, chitinases can inhibit fungal growth and development, often working in combination with other defense proteins like β-1,3-glucanases to provide more robust protection.

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**178.** (C) Protoplasts
PEG-mediated transformation is a chemical method used for the direct delivery of DNA into plant cells. This technique is typically applied to protoplasts, which are cells that have had their cell walls removed. Polyethylene glycol helps to destabilize the plasma membrane and facilitates the uptake of DNA from the surrounding solution. While effective for a wide range of species, it requires a reliable system for regenerating whole plants from the treated protoplasts.

**179.** (A) Monocot species
The rice actin-1 promoter is a robust and widely used constitutive promoter specifically preferred for genetic engineering in monocotyledonous plants. While the CaMV 35S promoter is very effective in dicots, it often shows lower and more variable activity in monocots like rice and maize. The actin-1 promoter provides stable, high-level transgene expression across nearly all tissues of the monocot plant, making it a reliable choice for cereal biotechnology.

**180.** (A) I, II, III and V
Reporter genes are versatile tools used to verify successful gene expression and study promoter activity. The gfp gene is unique because it allows for non-destructive, in vivo visualization of fluorescence. In contrast, the gusA assay is typically destructive, requiring the addition of a substrate that colors the tissue blue. The luc gene requires luciferin to produce light. These genes provide visual evidence of transformation but do not confer traits like herbicide resistance.

**181.** (A) To facilitate labeled nucleic acid probe binding
In the Southern blot procedure, DNA fragments must be denatured into single strands before they are transferred to the membrane. This is a critical step because the labeled nucleic acid probes used for detection can only hybridize to single-stranded DNA through base pairing. By breaking the hydrogen bonds between the two strands, the target sequence becomes accessible to the probe, allowing for the specific identification of the transgene among millions of genomic fragments.

**182.** (A) Antigen and antibody
The Enzyme-Linked Immunosorbent Assay is a widely used biochemical technique that relies on the highly specific interaction between an antigen and an antibody. In plant biotechnology, ELISA is frequently employed to detect and quantify the amount of a transgenic protein produced by a GM crop. A specific antibody is used to capture the transgenic protein from a sample, and a second, enzyme-linked antibody is used to generate a measurable signal.

**183.** (A) They differ in modes of action and cross-resistance.
Pyramiding a Cry protein with a Vegetative Insecticidal Protein is a highly effective strategy for delaying the development of insect resistance. These two classes of toxins bind to different receptors in the insect midgut and have distinct modes of action. Because of this, insects that develop resistance to a Cry protein are still likely to be susceptible to the Vip protein, and vice versa. This lack of cross-resistance makes it much harder for pests to adapt.

**184.** (A) Inhibiting digestive enzymes to cause starvation
Protease inhibitors are a natural defense mechanism that some plants use to combat insect pests. When an insect feeds on a transgenic plant expressing these inhibitors, the proteins bind to and neutralize the digestive proteases in the insect's midgut. This prevents the insect from breaking down and absorbing essential proteins from its food. This interference with nutrient digestion leads to severe growth inhibition, starvation, and eventual mortality of the feeding insect.

**185.** (A) Expressing mutant replicase to interfere with viruses
Replicase-mediated resistance is a form of pathogen-derived resistance where a transgenic plant expresses a portion of a virus's own replicase gene. Often, a mutated or non-functional version of the replicase protein is produced. This transgenic protein can then interfere with the assembly or function of the natural viral replication complex. By blocking the virus's ability to multiply its genome, the plant can effectively prevent the development and spread of the viral disease.

**186.** (A) They degrade two distinct structural wall components.
The co-expression of chitinase and β-1,3-glucanase in transgenic plants is a synergistic strategy for enhancing resistance to fungal

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specific allergenic motifs can indicate a potential risk that the protein might trigger an allergic response.

**152.** (C) Promoter regions and introns
A cDNA library is constructed from mRNA, meaning it represents only the genes that were actively expressed in the tissue at the time of sampling. Because mRNA is produced after the process of splicing, the cDNA sequences do not contain introns. Furthermore, as the process begins with the mature mRNA transcript, genomic elements such as promoters and other non-transcribed regulatory regions are also excluded from the library.

**153.** (C) Form a secretion channel for T-complex export
The Agrobacterium virB operon is a large cluster of genes that encodes the majority of the components for the Type IV secretion system. These proteins assemble into a complex, membrane-spanning channel that facilitates the transfer of the T-DNA complex and several other virulence proteins from the bacterium into the plant cell. This secretion channel is essential for the delivery of genetic material across both bacterial and plant membranes.

**154.** (B) They define the segment to be transferred.
The left and right border sequences are 25-base pair imperfect direct repeats that flank the T-DNA region on the Ti plasmid. These sequences are the only parts of the Ti plasmid that are strictly required for the mobilization of T-DNA. They serve as recognition sites for the VirD2 endonuclease, which nicks the DNA at these borders to define the exact segment that will be transferred into the plant genome.

**155.** (B) Biolistics (Particle bombardment)
Biolistics, or particle bombardment, is a physical method of direct gene transfer that uses high-velocity microcarriers to deliver DNA into plant cells. These microcarriers are typically made of dense metals like gold or tungsten and are coated with the DNA to be transferred. The particles are accelerated using helium gas or gunpowder to penetrate the cell wall and membrane, allowing the DNA to integrate into the plant genome.

**156.** (D) Seeds
The napin promoter is a well-characterized regulatory sequence derived from the napin gene of Brassica napus. It is a highly tissue-specific promoter that drives gene expression exclusively in the developing seeds. This makes it an invaluable tool for plant biotechnologists who wish to modify seed traits, such as oil composition or protein content, without affecting the growth or development of other parts of the plant.

**157.** (C) gusA
Screenable markers, like the gusA gene, are used to visually identify transformed cells or tissues rather than to provide a survival advantage. The expression of the gusA gene results in the production of an enzyme that can convert a colorless substrate into a bright blue product. This allows researchers to easily screen through many potential transformants and determine the spatial and temporal patterns of gene expression in various plant parts.

**158.** (A) One specific primer and a degenerate primer
TAIL-PCR is an efficient technique for amplifying unknown DNA sequences adjacent to a known region. Its main advantage is that it requires only one specific primer designed for the known sequence and a short, arbitrary degenerate primer. By using successive rounds of PCR with different annealing temperatures, the method selectively amplifies the flanking genomic DNA, providing a rapid way to identify transgene insertion sites or clone unknown promoters.

**159.** (C) Gel electrophoresis
In the Northern blot procedure, gel electrophoresis is a critical step used to separate RNA molecules based on their size. Total RNA is loaded onto a denaturing agarose gel and subjected to an electric field. Because smaller molecules move faster through the gel matrix than larger ones, the RNA forms a size-ordered gradient. This separation allows researchers to determine the size of a specific mRNA transcript after it is hybridized with a probe.

**160.** (A) P-II, Q-IV, R-III, S-I
Molecular techniques provide different metrics for analyzing transgenic plants. Southern blotting is used to determine transgene integration and copy number. Northern blotting detects the presence and size of specific mRNA transcripts. Western blotting is used to identify and quantify translated proteins. Finally, Quantitative Real-Time PCR provides a highly sensitive and exact measurement of the number of mRNA

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copies present in a sample, allowing for precise quantification.

**161.** (A) Areas planted with non-Bt versions of the crop
In the context of insect resistance management, a refuge is a designated area within or near a Bt crop field that is planted with non-transgenic versions of the same crop. The purpose of the refuge is to provide a habitat for a population of insects that have not been exposed to the Bt toxin. These susceptible insects can then mate with any rare resistant individuals, helping to maintain a high proportion of susceptible alleles in the population.

**162.** (C) By binding midgut receptors to form pores
Vegetative insecticidal proteins, such as Vip3A, are secreted by Bacillus thuringiensis during its growth phase and are highly effective against several lepidopteran pests. Like the better-known Cry proteins, Vip3A exerts its toxicity by binding to specific receptors on the midgut epithelium of susceptible insects. This binding leads to the formation of pores in the cell membrane, resulting in osmotic imbalance, cell lysis, and the eventual death of the insect.

**163.** (A) The viral sequence, leading to its degradation
RNA interference is a powerful tool for developing virus-resistant transgenic plants. The technology involves expressing a double-stranded RNA molecule that is identical in sequence to a part of the target virus's genome. This dsRNA is processed into small interfering RNAs that guide the plant's silencing machinery to specifically recognize and degrade the corresponding viral RNA. By targeting the viral sequence for destruction, the plant can effectively block viral replication and spread.

**164.** (B) Disrupting cell membrane integrity
Antimicrobial peptides like cecropins are an important part of the innate immune system in many organisms. These small, typically positively charged peptides work by interacting with the negatively charged lipids in the bacterial cell membrane. They insert themselves into the membrane and form pores, which disrupt its integrity and lead to the leakage of essential cellular contents. This rapid disruption of the membrane leads to the death of the bacterial pathogen.

**165.** (A) Drought-Responsive Element
The Dehydration-Responsive Element is a specific cis-acting DNA sequence found in the promoters of many genes that are induced by drought, cold, or high salinity. It serves as the binding site for the DREB class of transcription factors. When a plant experiences water stress, these transcription factors bind to the DRE site and activate the expression of a broad regulon of protective genes, helping the plant adapt to adverse environmental conditions.

**166.** (D) Has a vastly reduced affinity for glyphosate
The CP4 EPSPS gene, isolated from a soil bacterium, is widely used to develop glyphosate-tolerant crops. The enzyme encoded by this gene performs the same essential function in the shikimate pathway as the plant's natural EPSPS enzyme. However, the CP4 version has a unique structure that significantly reduces its affinity for the glyphosate molecule. This allows the enzyme to remain functional even in the presence of the herbicide, ensuring the plant's survival.

**167.** (A) Significantly reduced ethylene and delayed ripening
Ethylene is the primary gaseous hormone that regulates fruit ripening and softening. ACC oxidase is the final enzyme in the ethylene biosynthesis pathway, converting the precursor ACC into ethylene gas. By using antisense technology to suppress the expression of this enzyme in tomatoes, researchers can dramatically reduce the amount of ethylene produced. This results in a significant delay in the ripening process, allowing the fruit to stay firm for longer.

**168.** (A) Phytase
To improve the bioavailability of iron in biofortified crops, researchers often combine the expression of a ferritin gene with that of a phytase enzyme. While ferritin increases the total iron content, phytic acid in the seeds often binds to the iron and prevents its absorption by the human body. Expressing phytase helps to break down this anti-nutrient, releasing the iron and making it more readily available for absorption during digestion.

**169.** (B) To decrease linolenic acid to increase stability
Silencing the FAD3 desaturase gene is a strategy used to improve the quality of oil in crops like rapeseed and soybean. The FAD3 enzyme is responsible for converting linoleic

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acid into linolenic acid. By suppressing this gene, researchers can significantly reduce the concentration of linolenic acid in the seed oil. Because linolenic acid is highly susceptible to oxidation, reducing its levels increases the shelf-life and oxidative stability of the vegetable oil.

**170.** (A) Both A and R are true and R is the correct explanation of A.
Plastid transformation is known for its ability to achieve exceptionally high levels of transgenic protein accumulation. This is largely because each plant cell can contain hundreds to thousands of individual plastids, each with multiple copies of its genome. This results in a massive total gene copy number per cell. When a transgene is integrated into the plastid genome, it can be expressed at very high levels compared to nuclear transformation.

**171.** (A) siRNA production and mRNA cleavage
Post-transcriptional gene silencing is a natural defense and regulatory mechanism in plants that occurs after mRNA has been synthesized. It is primarily driven by the production of small interfering RNAs that are complementary to the target mRNA. These siRNAs guide the RNA-induced silencing complex to specifically recognize and cleave the homologous mRNA molecules. This sequence-specific degradation prevents the mRNA from being translated into a functional protein, effectively silencing the gene.

**172.** (B) 34-bp FRT sites
The FLP-FRT recombination system is a site-specific tool used for genome engineering in plants. The FLP recombinase enzyme specifically recognizes and binds to 34-base pair recognition sequences known as FRT sites. When two FRT sites are present in the genome, the FLP recombinase can mediate a recombination event between them. Depending on their orientation, this results in the excision or inversion of the DNA sequence located between the two sites.

**173.** (A) Environmental appraisal of large-scale GMO use
The Genetic Engineering Appraisal Committee is the primary regulatory body in India responsible for evaluating the environmental safety of genetically modified organisms. Its main function is to conduct a comprehensive appraisal of large-scale field trials and proposed commercial releases of GM crops. The committee reviews data on potential environmental risks, impacts on biodiversity, and food safety before granting any authorization for the commercial use of a transgenic product.

**174.** (A) Transgene movement to wild relatives
Analyzing gene flow is a critical component of the environmental risk assessment for genetically modified crops. Researchers evaluate the likelihood that a transgene, such as one conferring herbicide resistance or insect toxicity, could be transferred from the GM crop to wild relatives or neighboring conventional crops through cross-pollination. Understanding these patterns of gene movement is essential for managing the potential ecological impacts and maintaining the integrity of different agricultural systems.

**175.** (C) Differential display
Differential display is a powerful transcriptomics technique used to visualize and compare the patterns of gene expression in different biological samples. By using a series of specialized primers to amplify subsets of the mRNA population, researchers can generate a unique fingerprint of expressed genes. By comparing these patterns, they can identify specific mRNA bands that are present or absent under certain conditions, allowing for the discovery of differentially expressed genes.

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**176.** (B) VirC1
The VirC1 protein plays an important role in enhancing the efficiency of T-DNA transfer in Agrobacterium. It functions by binding to a specific DNA sequence called the “overdrive” element, which is located near the right border of the T-DNA on the Ti plasmid. This binding helps to facilitate the nicking and processing of the T-strand by the VirD2 endonuclease, significantly increasing the overall rate of T-DNA production and subsequent plant transformation.

**177.** (A) To facilitate recombination to join plasmids
In a co-integrative vector system, a region of DNA homology between the intermediate cloning vector and the Ti plasmid is essential for the transformation process. Because the intermediate vector cannot replicate independently in Agrobacterium, it must integrate into the Ti plasmid through a single crossover event at the site of homology. This recombination step creates a large, cointegrated plasmid that carries both the T-DNA and the virulence genes.

**126.** (B) Multiple transgene copies with sequence homology
Homology-dependent gene silencing is a regulatory process that is often triggered when multiple copies of a transgene, or sequences homologous to endogenous genes, are present in the plant genome. The plant perceives these high-copy or repetitive sequences as a potential threat, leading to the activation of silencing mechanisms. This can result in either the suppression of transcription or the sequence-specific degradation of the produced mRNA.

**127.** (A) Integration into the genome at unlinked loci
For the co-transformation strategy to be effective in producing marker-free plants, the selectable marker and the gene of interest must integrate into the plant genome at different, unlinked locations. This spatial separation is essential because it allows the two genes to segregate independently during the process of meiosis. By screening the progeny in the next generation, researchers can identify individuals that have retained the desired trait while losing the marker.

**128.** (C) Ministry of Environment, Forest and Climate Change
The Genetic Engineering Appraisal Committee is the apex regulatory body in India responsible for the environmental risk assessment and approval of genetically modified organisms. It operates under the aegis of the Ministry of Environment, Forest and Climate Change. The GEAC evaluates data from field trials and laboratory studies to ensure that any proposed commercial release of a GM crop is safe for the environment and human health.

**129.** (B) Beneficial insects like honeybees and beetles
When assessing the environmental impact of a Bt crop, it is crucial to determine whether the transgenic toxin has any unintended effects on non-target organisms. Regulatory frameworks typically require testing on a variety of beneficial insects that inhabit the agricultural landscape, such as honeybees, ladybird beetles, and lacewings. These studies help to ensure that the Bt protein is highly specific to the target pest and does not harm important ecological actors.

**130.** (A) VirD2 nicks the border → T-strand synthesis → VirE2 binds T-strand → Export via VirB/D4 complex
The processing and transfer of T-DNA in Agrobacterium follows a highly coordinated series of molecular events. First, the VirD2 endonuclease nicks the Ti plasmid at the border sequences. A single-stranded T-strand is then synthesized and coated with VirE2 proteins for protection. Finally, this T-complex is exported from the bacterial cell into the plant cell through a specialized secretion channel formed by the VirB and VirD4 proteins.

**131.** (A) Cloned fragments representing the whole genome
A genomic library is a comprehensive collection of cloned DNA fragments that ideally represents the entire genetic information of a specific organism. It is constructed by digesting the total genomic DNA with restriction enzymes and inserting the resulting fragments into suitable cloning vectors. These libraries are essential resources for researchers to isolate specific genes, study regulatory elements, and explore the overall organization of an organism's genome.

**132.** (B) The 5' end
During the Agrobacterium-mediated transformation process, the VirD2 protein remains covalently attached to the 5' end of the single-stranded T-DNA after the nicking reaction. This attachment serves multiple purposes, including protecting the 5' end from exonuclease degradation and acting as a nuclear localization signal. VirD2 plays a vital role in guiding the T-strand through the plant cell and into the nucleus for integration.

**133.** (A) Carbon and nitrogen
In a fascinating example of biological engineering, Agrobacterium tumefaciens transforms plant cells to produce opines, which are specialized molecules that the plant cannot use. However, the bacteria carry specific genes on their Ti plasmid that allow them to catabolize these opines. This creates a unique ecological niche where the transformed plant tissue provides a constant source of carbon and nitrogen exclusively for the infecting Agrobacterium population.

**134.** (D) Electric field strength and pulse duration
The efficiency of gene transfer via electroporation depends on the careful optimization of the

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pathogens. Chitin and β-1,3-glucans are the two major structural polysaccharides that make up most fungal cell walls. By targeting both components simultaneously, the enzymes can more effectively degrade the wall structure and inhibit fungal growth. this dual attack is much more potent than the action of either enzyme on its own.

**187.** (A) Proline
The enzyme delta-1-pyrroline-5-carboxylate synthetase catalyzes the rate-limiting step in the synthesis of proline from glutamate. Proline is a highly soluble amino acid that acts as a powerful osmoprotectant in plants. By overexpressing the P5CS gene, researchers can significantly increase the accumulation of proline in transgenic crops. This helps the cells maintain their osmotic balance and protects essential proteins and membranes from damage during periods of drought or high salinity stress.

**188.** (A) Aromatic amino acids
Glyphosate inhibits the EPSPS enzyme, which is a critical part of the shikimate pathway found in plants and many microorganisms. This metabolic pathway is responsible for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Because these amino acids are essential for protein synthesis and the production of many secondary metabolites, inhibiting their production leads to the rapid death of the plant, making it the target of glyphosate-based herbicides.

**189.** (A) Pseudomonas chloroaphis
The gene encoding the enzyme ACC deaminase, which is used in biotechnology to delay fruit ripening, was originally isolated from the soil bacterium Pseudomonas chloroaphis. This enzyme works by breaking down 1-aminocyclopropane-1-carboxylic acid, the immediate chemical precursor of the ripening hormone ethylene. By reducing the level of ACC in the plant tissues, the enzyme significantly slows down the production of ethylene gas, leading to an extension of fruit firmness and shelf-life.

**190.** (A) P-IV, Q-III, R-I, S-II
Specific genes are used to introduce various traits into transgenic crops. The psy gene is essential for β-carotene synthesis in Golden Rice. The bar gene provides resistance to glufosinate herbicides. For abiotic stress, the DREB1A transcription factor is used to enhance drought tolerance. In biotic stress management, the chitinase gene is commonly expressed to provide resistance against fungal pathogens by degrading their structural cell walls.

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**191.** (A) Terminating elongation at the 12-carbon stage
High-laurate canola was developed by introducing a gene encoding a specialized 12:0-acyl-carrier protein thioesterase from the California bay laurel. This enzyme plays a critical role in fatty acid biosynthesis by specifically recognizing and hydrolyzing 12-carbon acyl chains. This action terminates the fatty acid elongation process at the 12-carbon stage, preventing further growth and leading to a significant accumulation of lauric acid in the seeds of the transgenic plant.

**192.** (C) Homoplasmy
In plastid transformation, each plant cell initially contains a mixture of transformed and untransformed plastid genomes, a state known as heteroplasmy. Through multiple rounds of selection and regeneration, researchers aim to reach a state where every single copy of the plastid genome within a cell carries the transgene. This stable condition is called homoplasmy. Achieving homoplasmy is essential for the stable inheritance and consistent high-level expression of the transgenic trait.

**193.** (A) Formation of dsRNA and subsequent gene silencing
The integration of a transgene as inverted repeats in the plant genome can often lead to undesirable gene silencing effects. These repeats can pair with each other to form double-stranded RNA hairpins. The plant's internal defense machinery recognizes these dsRNAs and processes them into small interfering RNAs, which then guide the degradation of the corresponding mRNA. This post-transcriptional gene silencing effectively reduces or eliminates the expression of the intended transgenic trait.

**194.** (A) Induce recombinase expression when required
In site-specific recombination systems used for marker removal, it is often desirable to have precise control over when the recombination event occurs. Researchers frequently use inducible promoters, such as those responsive to heat-shock or specific chemicals, to drive the expression of the recombinase gene. By applying

the appropriate stimulus, they can activate the production of the recombinase only after the transgenic plant has been successfully selected and regenerated, ensuring efficient marker excision.

**195.** (C) IBSC
According to the biosafety guidelines in India, any researcher planning to initiate experiments involving recombinant DNA technology must first obtain approval from their Institutional Biosafety Committee. The IBSC is the first point of regulatory oversight and is responsible for ensuring that the proposed research is conducted safely, that appropriate containment levels are maintained, and that potential risks to the environment and human health are properly managed within the institution.

**196.** (A) Lab testing purified Bt protein on surrogate species
Tier-1 risk assessment for Bt crops involves conducting laboratory-based toxicity studies to evaluate potential impacts on non-target organisms. In these studies, purified Bt protein is fed to representative surrogate species, such as honeybees, ladybird beetles, or earthworms, at concentrations much higher than those found in the field. These high-dose tests provide a conservative estimate of risk, helping to determine whether the toxin has any unintended effects on beneficial or ecologically important species.

**197.** (A) Genetic linkage mapping and chromosomal walking
Map-based cloning is a forward-genetics approach used to isolate a gene based on its physical location in the genome. It relies on establishing tight genetic linkage between the target trait and molecular markers. Once a small genomic region is identified, researchers use chromosomal walking or jumping to move along the DNA until the specific gene is reached. This method is particularly useful for cloning genes for which the protein product is unknown.

**198.** (A) Inter-kingdom horizontal gene transfer
The natural process by which Agrobacterium tumefaciens transfers DNA into the plant genome is a remarkable example of inter-kingdom horizontal gene transfer. In nature, this bacterium has evolved a specialized system to deliver genetic material from its own Ti plasmid across both bacterial and plant membranes. This process results in the stable integration and expression of bacterial genes within the plant host, a phenomenon that has been adapted by biotechnologists for modern plant transformation.

**199.** (A) Auxin and cytokinin
In nature, Agrobacterium tumefaciens induces crown gall disease by transferring oncogenes into plant cells. These oncogenes encode enzymes involved in the overproduction of the plant hormones auxin and cytokinin. This hormonal imbalance triggers uncontrolled cell division and growth, leading to the formation of tumors. In biotechnology, these oncogenes are removed to "disarm" the Ti plasmid, allowing researchers to transfer desired genes without causing any abnormal growth in the transgenic plant.

**200.** (A) Both A and R are true and R is the correct explanation of A.
Particle bombardment is a highly versatile method for plant transformation because it can physically bypass many biological barriers. DNA-coated gold or tungsten microcarriers are accelerated to high velocities, allowing them to penetrate the rigid plant cell wall, the plasma membrane, and even the double membranes of organelles like plastids. This capability makes biolistics the preferred method for transforming the chloroplast genome, which is otherwise difficult to access using biological methods.

**201.** (A) Rubisco small subunit promoter
Tissue-specific promoters are essential for controlling where a transgene is expressed within a plant. The Rubisco small subunit promoter is a classic example of a tissue-specific promoter that is primarily active in green, photosynthetic tissues like leaves. It is regulated by light and ensures that the transgene product is accumulated only in the organs where it is needed, which is particularly useful for traits related to photosynthesis or localized pest defense.

**202.** (B) It confers a metabolic growth advantage on mannose.
The pmi selection system is a "positive selection" method because it provides transformed cells with a metabolic advantage rather than using a toxin to kill untransformed cells. The pmi gene encodes an enzyme that allows cells to convert mannose-6-phosphate into a usable form for

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glycolysis. In a medium where mannose is the sole carbon source, only the transformed cells containing the pmi gene can grow and regenerate, while untransformed cells remain dormant.

**203.** (A) It binds to unknown genomic sequences flanking the site.
In the TAIL-PCR technique, the Arbitrary Degenerate primer plays a crucial role in amplifying unknown DNA sequences. While specific primers bind to the known transgene sequence, the AD primer is designed to bind at multiple random locations within the surrounding genomic DNA. By using a series of specialized thermal cycles, the method favors the amplification of the specific segment between the known sequence and the nearest AD binding site, allowing researchers to recover flanking sequences.

**204.** (B) A reference/housekeeping gene
Quantitative Real-Time PCR is used to measure the relative abundance of mRNA transcripts in a sample. To ensure accurate comparisons across different samples, the data must be normalized against an internal control. This is typically a housekeeping gene, which is a gene that is expressed at a constant level across various tissues and experimental conditions. Normalization accounts for differences in the initial amount of RNA or the efficiency of the reverse transcription reaction.

**205.** (B) The acidic stomach destroys it; receptors are absent.
Bt proteins are considered safe for human consumption due to several physiological factors. These toxins are highly specific to the alkaline environment of an insect's midgut and are rapidly broken down by the high acidity of the human stomach. Furthermore, human intestinal cells lack the specific receptors that the toxins must bind to in order to exert their toxicity. This double layer of protection ensures that Bt proteins are non-toxic to mammals.

**206.** (A) A-amylase inhibitor
A-amylase inhibitors are specialized proteins used in genetic engineering to provide resistance against insect pests that rely on starch as their primary energy source. These inhibitors work by binding to the insect's α-amylase enzyme in the gut, preventing it from breaking down complex starches into simple, absorbable sugars. This interference with carbohydrate digestion effectively starves the insect, leading to significant inhibition of its growth, development, and overall survival rate.

**207.** (A) Inverted viral repeats designed to form a hairpin
RNA interference constructs for virus resistance are typically designed to produce a double-stranded RNA molecule within the plant cell. This is achieved by using inverted repeats of a target viral sequence, separated by a short spacer or intron. When transcribed, these repeats fold back to form a stable hairpin RNA. This dsRNA structure is recognized by the plant's silencing machinery, leading to the production of siRNAs that specifically target the virus for degradation.

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**208.** (B) The Hypersensitive Response (HR)
The Hypersensitive Response is a rapid and highly effective plant defense mechanism characterized by localized cell death at the site of pathogen infection. By sacrificing a few cells, the plant creates a physical barrier that restricts the nutrient supply and prevents the further spread of the pathogen to healthy tissues. This response is often triggered by the specific recognition of a pathogen effector protein by a corresponding resistance protein in the plant.

**209.** (A) Desiccation and dehydration
Late Embryogenesis Abundant proteins are highly hydrophilic proteins that accumulate in seeds during the final stages of development and in vegetative tissues during periods of water stress. They are thought to play a critical role in protecting cellular components from the damaging effects of desiccation. By acting as molecular chaperones and maintaining the integrity of proteins and membranes, LEA proteins help plants survive extreme dehydration and survive under severe drought conditions.

**210.** (D) I, II and III
Tolerance to the herbicide glyphosate can be achieved through several genetic engineering strategies. Glyphosate naturally targets the EPSPS enzyme in the shikimate pathway. One common approach is to overexpress a mutant version of the EPSPS enzyme, like CP4 EPSPS, which does not bind the herbicide. Additionally, the GOX gene can be introduced to directly degrade glyphosate into non-toxic products.

individuals that are heterozygous for a resistance allele are killed by the toxin. When combined with a refuge of non-Bt plants, this approach significantly delays the evolution of resistance in the target pest population.

**228.** (A) It binds sap-sucking insect midgut glycoproteins.
The GNA lectin is a valuable tool in plant biotechnology for controlling sap-sucking insects that are not susceptible to traditional Bt toxins. When these insects feed on a transgenic plant expressing the lectin, the protein binds to specific glycoproteins on the surface of their midgut epithelium. This binding interferes with normal digestion and nutrient absorption, leading to reduced growth and survival of the pests, making it an effective part of integrated pest management.

**229.** (B) Carries a significant transgenic mismatch mutation
RNA interference is a highly sequence-specific mechanism for gene silencing. For it to provide effective virus resistance, the small interfering RNAs produced by the transgenic plant must precisely match the sequence of the infecting virus. If the virus carries a significant mutation or mismatch in the targeted region, the siRNAs may fail to recognize and bind to the viral RNA. This specificity means that RNAi-mediated resistance can sometimes be overcome by highly variable or rapidly mutating viruses.

**230.** (A) I, III and IV
Genetic engineering for disease resistance utilizes various proteins and pathways. Overexpressing chitinases provides resistance to fungi by degrading their cell walls, while NPR1 acts as a master regulator to enhance broad-spectrum systemic resistance. Sarcotoxins are effective antibacterial peptides. However, phytoalexins are not pre-formed but are synthesized only after infection. Additionally, β-1,3-glucanase targets the structural components of fungal cell walls, rather than bacterial cell walls, to provide protection.

**231.** (A) Maintaining balance and protein/membrane stability
Proline is a multi-functional amino acid that acts as a powerful osmoprotectant in plants under stress. During drought or high salinity, plants accumulate high levels of proline to help maintain cellular osmotic balance. Beyond its role in osmoregulation, proline also acts as a molecular chaperone, stabilizing the structure of essential proteins and cell membranes. It can also help to scavenge reactive oxygen species, protecting the cell from oxidative damage during periods of environmental stress.

**232.** (C) Acetylating glufosinate to render it inactive
The bar gene provides resistance to the herbicide glufosinate by encoding the enzyme phosphinothricin acetyltransferase. Glufosinate works by inhibiting glutamine synthetase, leading to a toxic buildup of ammonia in the plant. The PAT enzyme detoxifies the herbicide by adding an acetyl group to its active component, phosphinothricin. This modification prevents the molecule from binding to its target enzyme, allowing the transgenic plant to maintain its metabolism and survive herbicide treatment during weed control.

**233.** (C) Ethylene
ACC synthase and ACC oxidase are the two most critical enzymes in the ethylene biosynthesis pathway in plants. ACC synthase converts S-adenosylmethionine into the intermediate ACC, which is then converted into ethylene gas by ACC oxidase. Because ethylene is the primary hormone that triggers fruit ripening and senescence, these enzymes are major targets for genetic manipulation. Suppressing either enzyme can dramatically reduce ethylene production, leading to a significant delay in ripening and an extension of shelf-life.

**234.** (A) Seed-specific promoter
To increase the iron content of a crop specifically in its edible parts, researchers typically drive the expression of the ferritin gene with a seed-specific promoter. This ensures that the iron-storage protein is produced and accumulated primarily within the developing grain endosperm, where it can provide the most nutritional benefit to consumers. Using a seed-specific promoter also avoids the potential metabolic costs and unintended effects of expressing ferritin in other tissues of the plant.

**235.** (B) Oleic acid to linoleic acid
The FAD2 gene encodes the enzyme omega-6 desaturase, which is responsible for introducing a double bond into oleic acid to convert it into linoleic acid. In plant biotechnology, silencing

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**219.** (D) Insertional mutagenesis via T-DNA
Insertional mutagenesis is a powerful tool in functional genomics that uses the Agrobacterium T-DNA as a mutagen. When the T-DNA integrates randomly into the plant genome, it can disrupt the function of an endogenous gene. By screening a large population of T-DNA tagged plants for specific phenotypes, researchers can identify which gene has been affected. Since the T-DNA sequence is known, it acts as a molecular tag that allows for the rapid cloning and identification of the disrupted gene.

**220.** (B) III only
The Agrobacterium vir regulon consists of several proteins that coordinate the transfer of T-DNA. VirA and VirG form a two-component signaling system that senses plant signals. VirB proteins assemble into the secretion channel, and VirD2 is the endonuclease that nicks the T-DNA borders and stays attached to the 5' end. However, it is the VirE2 protein, not VirD2, that coats the single-stranded T-strand along its entire length to protect it from degradation.

**221.** (B) T-DNA plasmid and separate helper vir plasmid
A binary vector system is the most common tool for Agrobacterium-mediated transformation. It consists of two separate plasmids that function together within the bacterial cell. One is a small, versatile binary vector that carries the T-DNA with the gene of interest. The second is a larger helper Ti plasmid that has been disarmed of its own T-DNA but still carries the virulence genes. These vir genes provide the necessary machinery in trans to transfer the T-DNA.

**222.** (C) Microinjection
Microinjection is a highly precise physical method of direct gene transfer. It involves using an extremely fine glass capillary and a micromanipulator to deliver a DNA solution directly into the nucleus or cytoplasm of a target cell. This technique allows for the transformation of individual cells, such as zygotes or microspores, with high accuracy. While effective, it is a labor-intensive and slow process that requires specialized equipment and a high degree of technical expertise.

**223.** (C) Light
The Rubisco small subunit promoter is a well-known plant promoter that is strongly regulated by light. It ensures that the genes involved in photosynthesis are expressed only when and where they are needed, primarily in the leaves and other green tissues exposed to sunlight. In biotechnology, this promoter is often used to drive the expression of transgenes that should be active during the day or specifically in the photosynthetic parts of the plant.

**224.** (A) Phosphomannose isomerase
The pmi gene, originally isolated from E. coli, encodes the enzyme phosphomannose isomerase. This enzyme is the basis for a positive selection system in plant transformation. Most plant cells cannot grow on mannose because they lack the ability to convert it into a usable carbohydrate. When the pmi gene is introduced and expressed, it allows the transformed cells to metabolize mannose, providing them with a selective growth advantage over the untransformed cells in the population.

**225.** (A) Labeled nucleic acid probe complementary to RNA
Northern blotting is a technique used to detect specific RNA sequences within a sample. Like Southern blotting, it relies on the principle of hybridization. After separating the RNA molecules by size through gel electrophoresis and transferring them to a membrane, researchers use a labeled nucleic acid probe. This probe is designed to be complementary to the target RNA transcript, allowing for the specific identification and quantification of gene expression at the transcriptional level.

**226.** (B) Lateral flow strip (Dipstick)
Lateral flow strips, often called dipsticks, are rapid and easy-to-use immunodiagnostic tools used in the field to detect transgenic proteins. These strips use antibodies specific to a target protein, such as a Bt Cry toxin, to provide a visual result within minutes. This method is highly effective for the quick screening of seeds or leaf samples to confirm the presence of a specific transgenic trait without the need for complex laboratory equipment or specialized training.

**227.** (D) 99%
In the context of insect resistance management, a "high dose" Bt crop is one that expresses the toxin at a concentration sufficient to kill at least 99% of the susceptible insect population. This strategy is designed to ensure that even

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efficient mobilization of genetic material from the bacterium into the plant cell.

**243.** (B) 25-bp imperfect direct repeats at the ends
The T-DNA region of the Ti plasmid is defined by the presence of two highly conserved 25-base pair sequences known as the left and right borders. These sequences are imperfect direct repeats that serve as the essential recognition sites for the bacterial virulence machinery. The VirD2 protein specifically recognizes these borders and nicks the DNA at these precise locations, defining the boundaries of the segment that will be transferred and integrated into the host plant's genome.

**244.** (A) Calcium chloride and spermidine
In the process of particle bombardment, DNA must be effectively coated onto the surface of the gold or tungsten microcarriers. This is typically achieved through a chemical precipitation process using calcium chloride and spermidine. These chemicals help to condense the DNA and promote its adherence to the metal particles. Ensuring a uniform and stable coating of DNA is critical for the successful delivery and subsequent integration of the transgene into the target plant cells during the bombardment.

**245.** (A) Localized insecticide expression at feeding sites
Wound-inducible promoters are valuable tools in plant biotechnology, especially for developing insect-resistant crops. These promoters are activated only when the plant tissue is physically damaged, such as during insect feeding. By driving the expression of an insecticidal gene with a wound-inducible promoter, the plant produces the toxin exactly where and when it is needed. This localized expression helps to minimize the metabolic cost to the plant and reduces the overall exposure of non-target organisms to the transgenic protein.

**246.** (A) Adding an acetyl group to phosphinothricin
The pat gene provides resistance to the herbicide glufosinate by encoding the enzyme phosphinothricin acetyltransferase. The herbicide works by inhibiting the enzyme glutamine synthetase, which is essential for nitrogen metabolism in plants. The PAT enzyme detoxifies the active ingredient of the herbicide, phosphinothricin, by adding an acetyl group to its chemical structure. This modification inactivates the herbicide, allowing the transgenic plant to maintain its vital metabolic processes and survive treatment, while weeds are successfully controlled.

**247.** (C) Cuts exactly once within the T-DNA sequence
To determine the exact copy number of a transgene using Southern blotting, researchers digest the genomic DNA with a restriction enzyme that has a single recognition site within the T-DNA. When the DNA fragments are separated and hybridized with a transgene-specific probe, each independent integration event will result in a unique band on the blot. By counting the number of bands, researchers can accurately determine how many copies of the gene have been integrated into the plant's genome.

**248.** (A) A higher amount of target mRNA in the sample
In Quantitative Real-Time PCR, the cycle threshold value represents the number of cycles required for the fluorescent signal to cross a predefined threshold. Because the amount of PCR product doubles in each cycle during the exponential phase, a lower Ct value indicates that the fluorescent signal was reached sooner. This directly correlates to a higher initial concentration of the target mRNA in the sample, providing a sensitive and reliable way to quantify the levels of gene expression.

**249.** (C) Lepidoptera
The cotton bollworm is a major agricultural pest that belongs to the insect order Lepidoptera. Transgenic Bt crops expressing the Cry1Ac toxin are specifically designed to target pests in this order. The Cry1 class of proteins is highly effective against lepidopteran larvae because they bind to specific receptors in their midgut. This high level of specificity ensures that the Bt crop provides effective pest control while having no impact on insects in other orders, such as beneficial pollinators or predators.

**250.** (A) It requires simultaneous evolution of resistance mechanisms.
Gene stacking, or pyramiding, is a superior strategy for insect resistance management because it makes it significantly more difficult for pests to adapt. By expressing multiple toxins with different modes of action in the same

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the FAD2 gene is a common strategy to increase the levels of oleic acid in seed oils. By blocking this conversion step, the metabolic pathway is diverted, leading to a significant accumulation of monounsaturated oleic acid, which is more stable and has various health benefits for human consumption.

**236.** (C) Allow for precise transgene integration via recombination
Chloroplast transformation offers a distinct advantage over nuclear transformation due to its reliance on homologous recombination for gene integration. Unlike the random integration that typically occurs in the nucleus, the use of flanking sequences homologous to the plastid genome allows for the extremely precise and predictable insertion of transgenes. This eliminates the "position effects" that often lead to variable gene expression in nuclear transformants and ensures that the transgene is integrated exactly where intended.

**237.** (A) Flanking the transgene with Matrix Attachment Regions
Transcriptional gene silencing can be a major challenge in creating stable transgenic plants. One effective strategy to mitigate this is to flank the transgene with Matrix Attachment Regions. These DNA sequences bind to the nuclear matrix, organizing the transgene into an independent chromatin loop. This physical arrangement helps to shield the gene from the silencing influence of surrounding heterochromatin and other nearby epigenetic signals, promoting more consistent and stable gene expression across different transgenic lines.

**238.** (A) Crossing lines expressing FLP and carrying FRT sites
The FLP-FRT recombination system is a powerful tool for removing selectable markers from transgenic plants. This process typically requires two independent transgenic lines: one containing the gene of interest and a marker flanked by FRT sites, and another expressing the FLP recombinase. By crossing these two lines, the FLP enzyme is brought into the same cell as the FRT sites in the hybrid progeny, where it then catalyzes the precise excision of the marker gene.

**239.** (A) Oversee institutional research within safety limits
The Institutional Biosafety Committee is a vital regulatory body that oversees all research involving recombinant DNA technology within a specific organization. Its primary role is to ensure that all experiments are conducted in compliance with national biosafety guidelines and that appropriate safety measures are in place to protect researchers, the public, and the environment. The IBSC reviews research proposals, inspects facilities, and monitors ongoing activities to ensure that any potential biological risks are effectively managed and mitigated.

**240.** (B) Mice or rats for acute oral studies
As part of the comprehensive safety assessment for a new genetically modified crop, researchers conduct toxicity tests on the transgenic protein. These studies typically involve feeding the purified protein to small mammals, such as mice or rats, in acute oral toxicity trials. The goal is to determine if the protein has any adverse health effects at levels far exceeding those that would be encountered through normal consumption, providing essential data for assessing the overall safety of the GM food.

**241.** (B) Enrich transcripts expressed in only one specific sample
Subtractive hybridization is a molecular technique used to isolate genes that are uniquely expressed in a particular tissue or under specific conditions. The process involves hybridizing two different mRNA populations and removing the sequences that they have in common. This results in a "subtracted" pool of cDNA that is highly enriched for the transcripts that are specific to the target sample. This method is particularly valuable for identifying genes involved in complex processes like development or stress responses.

**242.** (A) VirD1
The processing of T-DNA in Agrobacterium is initiated by the coordinated action of the VirD1 and VirD2 proteins. VirD1 functions as a topoisomerase that helps to relax the supercoiled DNA of the Ti plasmid, making the T-DNA border sequences accessible. Once the DNA is relaxed, VirD2 acts as a site-specific endonuclease to nick the borders, defining the T-strand. Both proteins are essential for the

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Combining these methods provides robust and effective herbicide resistance in transgenic crops.

**211.** (B) Tomato
The Flavr Savr tomato was a landmark in plant biotechnology as the first genetically engineered food crop to be approved for commercial sale. It was designed to have a longer shelf-life by using antisense technology to suppress the expression of polygalacturonase, an enzyme that softens fruit cell walls. While it was eventually withdrawn from the market due to various business and logistical challenges, it paved the way for future developments in food biotechnology.

**212.** (C) Maize
Golden Rice 2 is an improved version of the original biofortified rice, designed to accumulate significantly higher levels of pro-vitamin A in the endosperm. The breakthrough was achieved by replacing the original daffodil phytoene synthase gene with a much more efficient version isolated from maize. This change, combined with the original bacterial crtI gene, resulted in a dramatic increase in β-carotene content, providing more potential for addressing vitamin A deficiency in vulnerable populations.

**213.** (C) It is more oxidatively stable and healthier for human consumption.
High-oleic oils are highly valued in the food industry because they are much more oxidatively stable than oils containing high levels of polyunsaturated fats. This stability makes them ideal for high-temperature applications like commercial frying, as they do not break down easily or produce harmful compounds. Additionally, oleic acid is a monounsaturated fat that is considered healthier for cardiovascular health, making these transgenic oils a desirable alternative to traditional vegetable oils.

**214.** (D) aadA (confers spectinomycin resistance)
In plastid transformation, the aadA gene is the most widely used selectable marker. It encodes an enzyme that provides resistance to the antibiotics spectinomycin and streptomycin. This selection system is particularly effective for chloroplasts because these antibiotics inhibit the prokaryotic-like ribosomes found within the organelle. By including the aadA gene in the transformation vector, researchers can specifically select for cells where the chloroplast genome has successfully integrated and expressed the transgene.

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**215.** (C) Cytosine
Cytosine methylation is a fundamental epigenetic modification that plays a major role in gene regulation and silencing in plants. In the context of transgene expression, the addition of a methyl group to cytosine residues within the promoter sequence often leads to transcriptional gene silencing. This modification alters the local chromatin structure and prevents the binding of transcription factors, effectively turning off the gene without changing the underlying DNA sequence of the transgenic construct.

**216.** (B) T1 or subsequent segregating generations
When using the co-transformation strategy, the selectable marker and the gene of interest are initially integrated together in the T0 plant. Because they are often at different genomic locations, they will segregate independently during the formation of seeds. The actual identification of marker-free plants occurs in the T1 or subsequent generations. Researchers use molecular techniques to screen the progeny and find individuals that have inherited the desired trait but have lost the selectable marker gene.

**217.** (A) Safe handling, transport, and use of modified organisms
The Cartagena Protocol on Biosafety is an international treaty that governs the movements of living modified organisms from one country to another. It was established to ensure that the international trade of GMOs is conducted safely and does not pose a threat to biological diversity or human health. The protocol focuses on procedures for prior informed agreement and requires comprehensive risk assessments for the handling, transport, and use of all modified organisms.

**218.** (D) BRL-I trials
In the Indian biosafety regulatory framework, the evaluation of a new genetically modified crop occurs in distinct stages of field trials. Biosafety Research Level II trials are more extensive than the initial BRL-I trials and are used to collect more comprehensive data on agronomic performance and environmental safety. Permission to conduct BRL-II trials is only granted after a thorough review and successful completion of the initial BRL-I phase by the regulatory committees.

plant, the strategy requires an insect to develop multiple independent resistance mutations at the same time to survive. The statistical probability of such rare events occurring simultaneously is extremely low, which helps to maintain the effectiveness of the transgenic trait over a much longer period.

**********

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(D) Subcellular localization and amino acid composition

2009

**18.** In metabolomics, Gas Chromatography-Mass Spectrometry (GC-MS) is best suited for the analysis of which class of compounds?
(A) Highly polar, high molecular weight proteins
(B) Intact chromosomal DNA molecules
(C) Large, non-volatile complex carbohydrates
(D) Volatile and thermally stable metabolites

2003

**19.** Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a core analytical technique in ionomics primarily because it offers:
(A) Sensitive, simultaneous quantification of multiple isotopes.
(B) Precise determination of secondary protein structures.
(C) Direct visualization of distinct cellular compartments.
(D) Analysis of volatile organic compounds emitted by plants.

2010

**20.** Match the comparative genomics terms in List I with their correct definitions in List II.
List I: Comparative Genomics Terms
P. Orthologues
Q. Paralogues
R. Xenologues
S. Macrosynteny
List II: Definitions
1. Genes derived from horizontal gene transfer
2. Conservation of gene order over large chromosomal segments
3. Homologous genes separated by a speciation event
4. Homologous genes separated by a gene duplication event
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-3, S-2

2012

**21.** In Marker-Assisted Backcrossing (MABC), "background selection" refers to the process of:
(A) Accelerating the recovery of the recurrent parent genome across non-target chromosomes.
(B) Selecting markers tightly linked to the target gene.
(C) Selecting individuals that possess the target gene phenotype.
(D) Selecting against crossovers immediately adjacent to the introgressed gene.

2001

**22.** Which of the following prediction models in Genomic Selection (GS) assumes that all marker effects are drawn from a single normal distribution with equal variance?
(A) BayesB Bayesian regression models
(B) Random Forest machine learning algorithms
(C) BayesA hierarchical Bayesian models
(D) RR-BLUP assuming equal marker variances

2008

**23.** In Genome-Wide Association Studies (GWAS), incorporating the Q-matrix into the statistical model is primarily intended to correct for:
(A) Rare allele frequency variations
(B) Population structure and stratification
(C) Linkage disequilibrium decay rates
(D) Epistatic interactions among loci

2014

**24.** Which type of molecular marker represents the amplification of sequences located strictly between adjacent microsatellite repeats?
(A) RAPD
(B) CAPS
(C) ISSR
(D) RFLP

2002

**25.** Which step in the RFLP protocol relies on capillary action or electrophoretic transfer to move DNA fragments onto a nitrocellulose or nylon membrane?
(A) Restriction digestion
(B) Pre-hybridization
(C) Autoradiography
(D) Southern blotting

2015

**26.** Why are RAPD markers often criticized in multi-laboratory collaborative genetic mapping projects?
(A) They are sensitive to minor PCR variations, causing poor reproducibility.
(B) They are too expensive to synthesize for large-scale genetic projects.
(C) They specifically require prior sequence information for primer design.
(D) They generate strictly co-dominant data which is difficult to analyze.

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(D) Doubled Haploids (DH)

2023

**9.** In linkage mapping, which mapping function accounts for double crossovers by assuming there is no crossover interference?
(A) Kosambi mapping function
(B) Morgan mapping function
(C) Lander-Green algorithm
(D) Haldane mapping function

2004

**10.** Read the given assertion and reason and select the correct option.
Assertion (A): The ratio of physical distance (bp) to genetic distance (cM) is constant across all regions of a chromosome.
Reason (R): Recombination events are non-randomly distributed, often showing suppression in heterochromatic regions such as centromeres.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**11.** In Bulked Segregant Analysis (BSA) for qualitative traits, the DNA pools are constructed based on which of the following criteria?
(A) Similar geographic origin of the parent lines
(B) Contrasting phenotypic expression of the target trait
(C) Random selection from the early $F_1$ generation
(D) Identical overall genetic background of the lines

2006

**12.** Composite Interval Mapping (CIM) offers an advantage over Simple Interval Mapping (SIM) by incorporating which of the following?
(A) Only unlinked markers as background controls
(B) Additional markers as covariates to control background genetic effects
(C) Single marker regression models without recombination frequencies
(D) Exclusively physical map coordinates instead of genetic map coordinates

2017

**13.** Which Next-Generation Sequencing (NGS) platform historically utilized pyrosequencing chemistry, relying on the release of pyrophosphate and subsequent light emission?
(A) 454 Life Sciences (Roche)
(B) Illumina HiSeq system
(C) Ion Torrent PGM system
(D) Pacific Biosciences (PacBio)

2015

**14.** During genome assembly, what is the primary purpose of scaffolding?
(A) To identify promoter regions and open reading frames.
(B) To physically break the genomic DNA into fragments.
(C) To order and orient contigs using paired-end information.
(D) To translate DNA sequences into putative protein sequences.

2017

**15.** In forward genetics, the standard workflow typically proceeds in which of the following directions?
(A) From known gene sequence to mutant phenotype discovery
(B) From protein structure to genomic synteny mapping
(C) From RNA interference to gene sequence characterization
(D) From mutant phenotype to identification of the causative gene

2014

**16.** Serial Analysis of Gene Expression (SAGE) quantifies transcript abundance by relying on which specific feature?
(A) The continuous sequencing of full-length native mRNA molecules.
(B) The competitive hybridization of two distinct fluorescently labeled samples.
(C) The generation of short sequence tags from the 3' ends.
(D) The specific binding of antibodies to nascent polypeptide chains.

2014

**17.** Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) separates proteins based on which two distinct biochemical properties?
(A) Hydrophobicity and post-translational modifications
(B) Isoelectric point (pI) and molecular weight
(C) Three-dimensional conformation and electrical charge

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# CHAPTER 12
# Molecular Markers and Genomics

2008

**1.** Which of the following best distinguishes a co-dominant molecular marker from a dominant molecular marker in a diploid organism?
(A) Co-dominant markers amplify only the mutant allele in a segregating population.
(B) Co-dominant markers can distinguish heterozygous individuals from homozygous dominant individuals.
(C) Co-dominant markers are exclusively based on RNA transcript hybridization.
(D) Co-dominant markers cannot be used for mapping biparental inheritance.

2018

**2.** During the Restriction Fragment Length Polymorphism (RFLP) workflow, what is the primary function of Southern blotting?
(A) To selectively amplify DNA fragments using random decamer primers.
(B) To ligate adapters to the sticky ends of restriction fragments.
(C) To directly visualize nucleotide transitions under UV light.
(D) To transfer restriction-digested DNA fragments from an agarose gel to a solid membrane.

2011

**3.** What is the standard length of primers typically used in Randomly Amplified Polymorphic DNA (RAPD) assays?
(A) 10 nucleotides
(B) 6 to 8 nucleotides
(C) 18 to 22 nucleotides
(D) 30 to 35 nucleotides

2014

**4.** In the Amplified Fragment Length Polymorphism (AFLP) technique, why is a combination of a rare cutter (e.g., EcoRI) and a frequent cutter (e.g., MseI) utilized?
(A) To ensure all resulting fragments are larger than 10 kilobases.
(B) To prevent the need for adapter ligation during the assay.
(C) To generate fragments of optimal size for amplification and to reduce the complexity of the restriction digest.
(D) To degrade highly repetitive centromeric DNA specifically.

2025

**5.** Which underlying molecular mechanism is primarily responsible for the high level of polymorphism observed in Simple Sequence Repeats (SSRs)?
(A) Slipped-strand mispairing during replication
(B) Homologous recombination occurring during meiosis
(C) Random transposon excision and integration events
(D) Error-prone non-homologous end joining pathways

2019

**6.** In Single Nucleotide Polymorphism (SNP) analysis, a substitution from Adenine to Guanine is classified as which of the following?
(A) Transversion mutation
(B) Frameshift mutation
(C) Inversion mutation
(D) Transition mutation

2009

**7.** Cleaved Amplified Polymorphic Sequences (CAPS) markers are generated by incorporating which specific step after PCR amplification?
(A) Sanger sequencing of the amplified fragment
(B) Restriction endonuclease digestion of the PCR product
(C) Ligation of fluorescent adapters to the blunt ends
(D) Hybridization with specific radio-labeled probes

NODIA

2021

**8.** Which of the following mapping populations requires the longest development time in crops due to the necessity of repeated selfing to achieve near-complete homozygosity?
(A) F2 population
(B) Backcross (BC) population
(C) Recombinant Inbred Lines (RILs)

2022

**27.** During the selective amplification step of AFLP, how do the selective primers differ from the pre-amplification primers?
(A) They lack the adapter-specific sequences.
(B) They contain 1 to 3 additional selective nucleotides at their 3' ends.
(C) They are radiolabeled with phosphorus-32 at the 5' end.
(D) They are synthesized purely from RNA nucleotides.

2012

**28.** EST-SSRs (Expressed Sequence Tag-SSRs) typically show lower polymorphism compared to genomic SSRs. What is the primary evolutionary reason for this?
(A) EST-SSRs are located in untranscribed regions.
(B) EST-SSRs are situated in highly conserved coding regions.
(C) Genomic SSRs replicate at a slower rate than EST-SSRs.
(D) EST-SSRs undergo higher rates of spontaneous transversions.

2002

**29.** KASP (Kompetitive Allele Specific PCR) genotyping technology depends on which of the following principles to detect SNPs?
(A) Competitive binding of two allele-specific forward primers and a reverse primer
(B) Endonuclease digestion of mismatched hybrids within the PCR reaction
(C) Direct Sanger sequencing of the target locus for every individual
(D) DNA microarrays hybridized with fluorescent probes on a solid surface

2011

**30.** Consider the following statements regarding locus-specific molecular markers:
I. SCAR markers are developed by sequencing a specific RAPD fragment and designing longer, specific primers.
II. CAPS markers require sequence information to identify restriction site variations within the amplified locus.
III. ISSR markers require prior sequence information of the target genome to design the repeat-anchored primers.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) III only
(C) II and III
(D) I only

2008

**31.** Which of the following populations is uniquely classified as "immortal" because it can be indefinitely propagated by seed without undergoing further genetic segregation?
(A) F2 population
(B) Backcross 1 (BC1)
(C) F3 families
(D) Recombinant Inbred Lines (RILs)

2007

**32.** To conclude statistically that two loci are genetically linked, the LOD (Logarithm of the Odds) score threshold is typically set at or above:
(A) 1.0
(B) 2.0
(C) 3.0
(D) 4.0

2014

**33.** The relationship between genetic distance (cM) and physical distance (bp) can drastically fluctuate. In which chromosomal region is the cM/Mb ratio typically the lowest?
(A) Telomeric regions
(B) Pericentromeric heterochromatin
(C) Euchromatic regions
(D) Gene-dense chromosomal arms

NODIA

2011

**34.** In the application of Bulked Segregant Analysis (BSA), what is the maximum recommended number of individuals that should typically be pooled in a single bulk to maintain the detection sensitivity of rare alleles linked to the trait?
(A) 2 to 4 individuals
(B) 10 to 20 individuals
(C) 100 to 150 individuals
(D) 500 to 1000 individuals

2011

**35.** Single Marker Analysis (SMA) for QTL mapping primarily suffers from which major statistical limitation?
(A) It confounds QTL magnitude with recombination frequency.
(B) It requires dense, whole-genome physical maps.
(C) It can only analyze dominant markers.
(D) It strictly limits the analysis to epistatic interactions.

2019

**108.** A transcriptomic analysis utilizing microarray technology depends fundamentally on which physical process?
(A) Complementary base-pairing between cDNA and immobilized probes
(B) Competitive enzymatic digestion
(C) Mass-to-charge ratio separation of ionized nucleic acids
(D) Ligation of short sequence tags into concatemers

2002

**109.** In MALDI-TOF Mass Spectrometry, "TOF" stands for Time-Of-Flight. This metric directly corresponds to the ionized peptide's:
(A) Mass-to-charge ($\frac{m}{z}$) ratio
(B) Molar absorptivity
(C) Three-dimensional conformational fold
(D) Isoelectric point

2003

**110.** Read the given assertion and reason and select the correct option.
Assertion (A): Gas Chromatography-Mass Spectrometry (GC-MS) often requires chemical derivatization of metabolites prior to analysis.
Reason (R): Derivatization is necessary to increase the volatility and thermal stability of polar metabolites.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**111.** High-throughput elemental profiling (ionomics) often explores mineral homeostasis. Which of the following elements would typically NOT be analyzed in a standard ionomics plant tissue profile?
(A) Zinc (Zn)
(B) Iron (Fe)
(C) Manganese (Mn)
(D) Helium (He)

2005

**112.** When comparing the genomes of Arabidopsis and Brassica, extensive regions displaying synteny are observed, though rearranged. Such large-scale conserved block arrangements are termed:
(A) Microsynteny
(B) Paralogous clustering
(C) Macrosynteny
(D) Epigenetic silencing

2012

**113.** Gene pyramiding is most strategically deployed in crop improvement to achieve which specific goal?
(A) Increasing the mutation rate of a susceptible allele
(B) Developing durable resistance by accumulating multiple R-genes
(C) Decreasing the ploidy level of a cultivar
(D) Removing all regulatory DNA sequences from the genome

2023

**114.** Unlike traditional Marker-Assisted Selection (MAS) which relies on a few significant markers, Genomic Selection (GS) utilizes:
(A) Only dominant RAPD markers across the entire population
(B) Strict phenotypic selection methods ignoring molecular data
(C) Exclusively cytogenetic banding patterns for chromosome analysis
(D) All genome-wide markers used to calculate breeding values

2013

**115.** Linkage Disequilibrium (LD) decay refers to the statistical decline in non-random association between alleles at different loci. What causes LD to decay over generations?
(A) Meiotic recombination
(B) Genetic drift exclusively
(C) Somatic mutations
(D) Transcriptional silencing

2003

**116.** Which specific inheritance pattern restricts the utility of chloroplast DNA markers for tracking pollen-mediated gene flow in most angiosperms?
(A) They are dominantly inherited.
(B) They undergo rapid horizontal gene transfer.
(C) They exhibit strictly maternal inheritance.
(D) They exhibit strictly biparental inheritance.

2011

**117.** During RFLP analysis, what is the specific consequence of a point mutation that creates a new, novel restriction site within a previously intact DNA fragment?
(A) The large fragment is cleaved into two smaller ones, altering banding.
(B) The probe will no longer hybridize to the DNA.

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single genotype.
(C) To induce novel mutations in the target genome.
(D) To map the exact physical location of a centromere.

2015

**45.** In Genomic Selection, the "training population" is uniquely characterized by having been evaluated for:
(A) Both high-density markers and precise phenotypic data
(B) Only genotypic data across the genome
(C) Only phenotypic performance for the target trait
(D) Neither genotypes nor phenotypes; it relies on simulated data

2012

**46.** The rate of Linkage Disequilibrium (LD) decay in a specific crop species heavily influences which parameter in a Genome-Wide Association Study (GWAS)?
(A) The type of restriction enzyme used for CAPS markers
(B) The ratio of transitions to transversions
(C) The marker density required to identify significant associations
(D) The physical size of the gel used for electrophoresis

2024

**47.** Which feature restricts the utility of standard hybridization-based markers like RFLPs for high-throughput genotyping?
(A) They are co-dominant markers.
(B) They fail to detect insertion-deletion (InDel) variations.
(C) They are strictly limited to coding sequences.
(D) They demand large DNA quantities and time-consuming procedures.

2006

**48.** In the Southern blotting procedure, what is the role of alkaline transfer buffer (e.g., NaOH)?
(A) To digest the DNA at specific palindromic sites
(B) To neutralize the gel for subsequent staining
(C) To denature double-stranded DNA into single strands
(D) To permanently cross-link DNA to the agarose matrix

2017

**49.** Due to their random amplification nature, RAPD profiles can contain artifact bands. Which strategy is most commonly recommended to mitigate this issue?
(A) Lowering the annealing temperature to 25°C
(B) Standardizing strict PCR cycling and template concentrations
(C) Running the PCR for only 10 cycles
(D) Using single-stranded RNA as the starting template

NODIA

2011

**50.** Consider the following statements concerning the Amplified Fragment Length Polymorphism (AFLP) technique:
I. AFLP utilizes restriction enzymes to fragment the genomic DNA.
II. Adapters are ligated to the sticky ends of the restricted DNA fragments.
III. Selective amplification relies on primers extending into the restriction fragments by 1-3 nucleotides.
IV. AFLP strictly generates co-dominant marker data.
V. The technique does not require prior knowledge of the target DNA sequence.
Which of the above statements are correct?
(A) I, II, III and V
(B) II, III and IV
(C) I, IV and V
(D) I, II and IV

2002

**51.** Which DNA polymerase property is fundamentally required for the mechanism of replication slippage, leading to SSR length polymorphisms?
(A) Frequent dissociation and misalignment at tandem repeats
(B) 5' to 3' exonuclease activity
(C) High fidelity proofreading at transition sites
(D) Strict reliance on RNA primers for Okazaki fragment initiation

2001

**52.** TaqMan assays achieve high specificity in SNP genotyping through the action of which specific enzymatic property?
(A) 3' to 5' exonuclease activity of T4 DNA polymerase
(B) DNA ligase sealing a nicked strand
(C) 5' to 3' exonuclease activity of Taq DNA polymerase
(D) Reverse transcriptase extending a bound RNA probe

2014

**53.** To find Sequence Characterized Amplified Region (SCAR) markers from a RAPD band, the standard protocol begins with:
(A) Isolating the RAPD fragment, cloning, and sequencing it.
(B) Synthesizing random decamer primers based on the species' GC content.
(C) Subjecting the RAPD product to complete digestion by restriction enzymes.
(D) Performing a Southern blot of the RAPD gel and probing with SSR motifs.

2018

**54.** A researcher requires a mapping population where every individual is fully homozygous at all loci. Which population should they develop?
(A) Backcross population ($BC1F_1$)
(B) F2 population
(C) F3 bulk population
(D) Doubled Haploids (DH)

2009

**55.** If the recombination fraction between two genes is 0.15, what is the approximate map distance between them in centimorgans (cM) assuming the Haldane mapping function?
(A) 15.0 cM
(B) 17.8 cM
(C) 16.4 cM
(D) 19.2 cM

2001

**56.** Cytogenetic mapping localizes genes or markers to specific physical regions of a chromosome utilizing which primary methodology?
(A) Measurement of recombination frequencies
(B) Gas chromatography-mass spectrometry
(C) Fluorescence in situ hybridization (FISH)
(D) Whole-genome shotgun sequencing

2002

**57.** When executing Bulked Segregant Analysis (BSA) in an F2 population for a newly discovered disease resistance trait, DNA is extracted and pooled from which specific individuals?
(A) Randomly selected individuals from the entire F2 population
(B) Only the original susceptible parent lines
(C) Only heterozygous individuals identified by a progeny test
(D) Two groups representing extremes of the phenotypic distribution

2023

**58.** Interval Mapping (IM) in QTL analysis utilizes a statistical framework to test for the presence of a QTL at regular intervals between adjacent markers. Which statistical approach is predominantly used?
(A) Maximum Likelihood estimation
(B) Fisher's Exact Test
(C) Chi-square goodness-of-fit
(D) Pearson correlation coefficient

2019

**59.** The Whole Genome Shotgun (WGS) sequencing approach relies heavily on:
(A) Prior physical mapping of the genome into an ordered BAC library.
(B) High-throughput sequencing of random fragments and algorithmic assembly.
(C) Targeted sequencing of strictly centromeric regions.
(D) Sanger sequencing of expressed sequence tags (ESTs) only.

2007

**60.** Read the given assertion and reason and select the correct option.
Assertion (A): Identifying Open Reading Frames (ORFs) is generally simpler and more accurate in prokaryotic genomes compared to eukaryotic genomes.
Reason (R): Prokaryotic genes are typically contiguous and lack introns, whereas eukaryotic genes frequently contain complex exon-intron structures.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**61.** Agrobacterium tumefaciens-mediated T-DNA insertion is a powerful tool in functional genomics primarily used to generate:
(A) Random insertional mutant libraries
(B) Targeted RNAi knockdowns
(C) High-density linkage maps
(D) Purified secondary metabolites

2018

**62.** In modern high-throughput RNA-Seq pipelines, the metric "TPM" is standardly used to report transcript expression levels. TPM stands for:
(A) Total Protein Molarity
(B) Tag Proportion Metric

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(C) Transcripts Per Million
(D) Targeted Primer Multiplexing

2023

**63.** Which analytical tool is considered the gold standard for identifying the precise amino acid sequence of a peptide following 2D-PAGE separation?
(A) Western blotting
(B) Edman degradation alone
(C) Yeast two-hybrid screening
(D) Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS)

2010

**64.** A metabolomics researcher applies Liquid Chromatography-Mass Spectrometry (LC-MS) instead of GC-MS. What is the most likely reason for this choice?
(A) The target metabolites are highly volatile.
(B) The sample consists exclusively of inert gases.
(C) The target metabolites are polar, non-volatile, and thermally labile.
(D) LC-MS is strictly utilized for genomic sequencing.

2025

**65.** The study of mineral nutrient homeostasis in crops under salt stress through multi-elemental profiling is a direct application of:
(A) Transcriptomics
(B) Ionomics
(C) Metagenomics
(D) Epigenomics

2005

**66.** Comparative genomics reveals that the order of genes along a chromosome is largely conserved between rice and wheat. This structural conservation is termed:
(A) Genetic drift
(B) Epistasis
(C) Horizontal gene transfer
(D) Collinearity

2025

**67.** During recombinant selection in MAS, the primary goal is to select individuals that exhibit:
(A) Cross-over events flanking the target locus to minimize linkage drag.
(B) High levels of heterozygosity across all linkage groups.
(C) Maximum background contribution from the donor parent.
(D) Phenotypic traits unrelated to the introgressed locus.

2010

**68.** Genomic Estimated Breeding Values (GEBVs) in Genomic Selection are computed by:
(A) Simply adding the phenotypic scores of the parents.
(B) Evaluating the segregation ratio of a single major QTL.
(C) Summing predicted effects of all genome-wide markers simultaneously.
(D) Counting the total number of chromosomes in the genotype.

2017

**69.** In a GWAS analysis for yield traits in maize, omitting the kinship matrix (K-matrix) correction from the mixed linear model will most likely lead to:
(A) False-positive associations due to relatedness
(B) Increased precision in measuring LD decay
(C) Total failure of the marker genotyping platform
(D) Immediate identification of causal epistatic interactions

NODIA

2006

**70.** Match the molecular marker acronyms in List I with their characteristic features in List II.
List I: Molecular Marker Acronyms
P. RFLP
Q. RAPD
R. SSR
S. SNP
List II: Characteristic Features
1. Highly abundant, single-base variation, bi-allelic
2. Utilizes short arbitrary decamer primers
3. Based on restriction digestion and probe hybridization
4. Based on tandem repeat length variations, multi-allelic
(A) P-3, Q-2, R-4, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-1, Q-4, R-3, S-2

2024

**71.** When comparing hybridization-based markers to PCR-based markers, which of the following is considered a primary advantage of PCR-based markers?
(A) They consistently detect exclusively dominant alleles.
(B) They require significantly lesser amounts of

nucleotide level.
IV. Xenologous genes are the result of horizontal gene transfer between different species.
Which of the above statements are correct?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I, II and IV

2009

**91.** In the context of Marker-Assisted Selection (MAS), what defines "foreground selection"?
(A) The selection of markers distributed across the non-target genome.
(B) Using markers tightly linked to target genes to identify carriers.
(C) The phenotypic evaluation of traits under field conditions.
(D) The elimination of dominant markers from the dataset.

2015

**92.** Training population optimization in Genomic Selection aims to maximize prediction accuracy. Which factor is most crucial in selecting the training population?
(A) High genetic relatedness and linkage disequilibrium with the breeding population.
(B) It must be completely genetically unrelated to the breeding population.
(C) It must consist entirely of mutant lines.
(D) It must be evaluated exclusively in a single environment.

2023

**93.** Genome-Wide Association Studies (GWAS) exploit historical recombination events within natural populations. This results in mapping resolution that is typically:
(A) Higher than that of standard biparental linkage mapping.
(B) Lower than that of biparental linkage mapping.
(C) Equivalent to simple F2 segregation mapping.
(D) Restricted to only the centromeric regions.

2013

**94.** A marker segregates in an F2 population (derived from two homozygous parents) at a strict 3:1 ratio (presence vs. absence of band). This mathematical segregation proves the marker is:
(A) Co-dominant
(B) Epistatic
(C) Dominant
(D) Pleiotropic

2002

**95.** RFLP relies on sequence variation altering restriction enzyme recognition sites. What type of mutation primarily causes the loss of a restriction site?
(A) Silent mutation in a downstream intron
(B) Complete genome duplication
(C) Alternative splicing
(D) Substitution within the recognition palindrome

2019

**96.** What is the fundamental mechanism allowing RAPD primers to amplify discrete DNA fragments from genomic DNA?
(A) Specific binding to poly-A tails of mRNA.
(B) Continuous amplification of repetitive satellite DNA only.
(C) Binding to closely spaced, inverted complementary sequences.
(D) Endonuclease cleavage creating staggered sticky ends.

2007

**97.** During AFLP analysis, the pre-amplification PCR step utilizes primers with typically how many selective nucleotides at their 3' ends?
(A) Zero
(B) One
(C) Three
(D) Five

2014

**98.** Why are dinucleotide repeats (e.g., (CA)n) generally more prone to replication slippage than hexanucleotide repeats?
(A) DNA polymerase possesses specific exonucleases for hexanucleotides.
(B) Dinucleotides form stable secondary hairpin structures that prevent slippage.
(C) Dinucleotides are strictly located in centromeres where replication is suppressed.
(D) Short repetitive dinucleotides misalign more easily during replication.

2003

**99.** Which statement best describes a fundamental limitation of high-throughput SNP arrays (SNP Chips)?
(A) They are afflicted by ascertainment bias by only genotyping known SNPs.
(B) They can only screen a few dozen markers simultaneously.
(C) They require hazardous radioisotopes for

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(A) Linkage drag from unlinked chromosomes
(B) False-positives due to random sampling errors
(C) Epigenetic silencing of the pooled DNA
(D) Over-digestion of the DNA by restriction enzymes

2001

**82.** Which QTL mapping methodology explicitly models the presence of multiple QTLs simultaneously to reduce residual variance and improve detection power?
(A) Single Marker Analysis (SMA)
(B) Simple Interval Mapping (SIM)
(C) Two-point Linkage Analysis
(D) Multiple Interval Mapping (MIM)

2011

**83.** The major advantage of utilizing Next-Generation Sequencing (NGS) over classical Sanger sequencing for whole-genome sequencing is:
(A) Massive parallelization of sequencing reactions to increase throughput.
(B) The generation of much longer read lengths per sequence.
(C) The elimination of bioinformatics assembly tools.
(D) The absolute zero error rate in raw sequence reads.

2011

**84.** In bioinformatics genome annotation, what is an "Open Reading Frame" (ORF)?
(A) A sequence of DNA bounded by restriction enzyme sites.
(B) The promoter region where RNA polymerase binds.
(C) A codon sequence bounded by a start and stop codon.
(D) The highly repetitive DNA found at telomeres.

2025

**85.** Reverse genetics approaches aim to determine the function of a specific gene. Which of the following is a classic reverse genetics technique?
(A) Generating gene knockouts using the CRISPR-Cas9 system
(B) Screening a natural population for drought-tolerant phenotypes
(C) Performing a standard diallel cross
(D) Scoring RFLP markers in an F2 population

2025

**86.** Which application is an RNA-Seq pipeline specifically designed to perform?
(A) High-throughput quantification of protein expression levels
(B) Identification of genomic single nucleotide polymorphisms via TaqMan
(C) Analysis of elemental ion profiles in root tissues
(D) Comprehensive characterization and quantification of transcriptomes

2023

**87.** In the Yeast Two-Hybrid system, a functional transcription factor is reconstituted when interacting proteins bring together which two specific domains?
(A) The restriction and modification domains
(B) The DNA-binding domain (DBD) and activation domain (AD)
(C) The kinase and phosphatase domains
(D) The helicase and polymerase domains

2023

**88.** A major challenge in global metabolomics profiling is the vast chemical diversity of metabolites. How is this challenge typically addressed analytically?
(A) By utilizing only one extraction solvent to normalize the data
(B) By amplifying the metabolites using PCR prior to detection
(C) By employing a suite of orthogonal analytical platforms
(D) By strictly limiting the analysis to volatile compounds

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2004

**89.** Which physiological process is most directly elucidated through the application of ionomics?
(A) Splicing of pre-mRNA in the nucleus
(B) Folding of nascent polypeptides
(C) Retrotransposon mobilization
(D) Regulation of mineral nutrient uptake mechanisms

2024

**90.** Consider the following statements concerning comparative genomics:
I. Paralogous genes originate via gene duplication events within a single lineage.
II. Orthologous genes originate via speciation events and typically retain the same biological function.
III. Macrosynteny refers to gene order conservation visible strictly at the single-

template DNA.
(C) They eliminate the need for oligonucleotide synthesis.
(D) They inherently bypass the need for electrophoresis.

2010

**72.** Which component is strictly necessary for visualizing target DNA sequences on an autoradiograph during RFLP analysis?
(A) Ethidium bromide
(B) Fluorescent intercalating dye
(C) Radioisotope-labeled probe (e.g., $^{32}P$)
(D) UV transilluminator

2016

**73.** Which of the following factors is LEAST likely to cause reproducibility issues in a RAPD assay?
(A) Minor fluctuations in the thermal cycler ramp rate
(B) Differences in the concentration of magnesium chloride in the buffer
(C) Use of DNA polymerases from different manufacturers
(D) The length of the genomic DNA extraction protocol

2004

**74.** In the standard AFLP procedure, MseI restriction sites are amplified more frequently than EcoRI restriction sites. Why?
(A) MseI is a 4-base cutter generating small fragments compared to EcoRI.
(B) EcoRI targets RNA exclusively.
(C) The selective primers only bind to the MseI adapters.
(D) MseI degrades heterochromatin faster than EcoRI.

2010

**75.** Primers designed for the amplification of SSR markers must be complementary to:
(A) The repetitive motif itself (e.g., strictly to the (CA)n repeats).
(B) Conserved flanking regions uniquely surrounding the SSR locus.
(C) Random decamer sequences distributed globally in the genome.
(D) Centromeric satellite repeats only.

2009

**76.** SNP Chips (microarrays) for high-throughput genotyping function primarily based on which molecular interaction?
(A) Differential centrifugation of genomic fragments
(B) Allele-specific oligonucleotide hybridization
(C) Endonuclease digestion at palindromic sites
(D) Gel filtration chromatography

2008

**77.** Inter-Simple Sequence Repeat (ISSR) markers generate polymorphisms without requiring prior sequence knowledge. This is achieved by anchoring the primer to:
(A) The 3' or 5' end of a microsatellite repeat sequence.
(B) The initiation codon of highly expressed genes.
(C) Random hexamer sequences found in introns.
(D) Telomeric repetitive DNA only.

2005

**78.** In developing Near Isogenic Lines (NILs) through repeated backcrossing, which generation is typically considered sufficient to recover >99% of the recurrent parent genome?
(A) F2
(B) BC1
(C) BC2
(D) BC6

2014

**79.** Linkage mapping critically depends on analyzing the frequency of which specific cellular event?
(A) Mitotic non-disjunction
(B) Transposon excision
(C) DNA methylation
(D) Meiotic crossing-over

2017

**80.** Consider the following statements regarding genetic and physical maps:
I. Genetic map distances are measured in base pairs (bp).
II. Physical maps reflect the actual physical length of DNA sequences.
III. The ratio of genetic distance to physical distance remains constant across the entire chromosome.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II and III
(C) I and III
(D) III only

2025

**81.** Which statistical phenomenon can complicate the identification of closely linked markers in Bulked Segregant Analysis (BSA) when the bulk size is too small?

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signal detection.
(D) They cannot differentiate between homozygous and heterozygous states.

2021

**100.** Consider the following statements regarding mapping populations:
I. Backcross populations are primarily used for introgressing a single trait from a donor to a recurrent parent.
II. RIL populations capture only a single generation of meiotic recombination.
III. F2 populations provide a high level of heterozygosity.
IV. Doubled Haploid lines are perfectly homozygous across all loci.
V. Near Isogenic Lines (NILs) differ strictly at the entire mitochondrial genome level.
Which of the above statements is/are INCORRECT?
(A) II and V
(B) I, III and IV
(C) II, IV and V
(D) I and II

2024

**101.** Recombination frequency ($\theta$) rarely exceeds 0.5 (or 50%). Why does this mathematical limit exist?
(A) Independent assortment of unlinked genes yields 50% recombinant gametes.
(B) Because chromosomes can only break once per meiosis.
(C) Because mapping functions fail mathematically at values above 0.5.
(D) Because double crossovers perfectly cancel out single crossovers.

2009

**102.** What happens to the cM to physical bp ratio in centromeric heterochromatin regions compared to distal euchromatic regions?
(A) It drastically increases because recombination is highly frequent.
(B) It remains mathematically identical.
(C) It becomes infinite as there is no DNA in centromeres.
(D) It drastically decreases because recombination is heavily suppressed.

2023

**103.** In Bulked Segregant Analysis (BSA) combined with Next-Generation Sequencing (QTL-seq), what specific metric is plotted across the genome to identify the target genomic region?
(A) The absolute length of the assembled contigs
(B) The SNP-index (allele frequency difference) between contrasting pools
(C) The total molarity of extracted DNA
(D) The RNA transcription rate

2022

**104.** Which of the following is a primary disadvantage of Single Marker Analysis (SMA) for QTL mapping?
(A) It strictly requires completely sequenced genomes.
(B) It necessitates the simultaneous computation of hundreds of markers in a single matrix.
(C) It cannot distinguish tightly linked small-effect QTLs from loose large-effect QTLs.
(D) It is only applicable to qualitative Mendelian traits.

2024

**105.** The primary limitation of Next-Generation Sequencing (e.g., short-read Illumina) in de novo genome assembly is:
(A) High per-base sequencing costs.
(B) Requirement of vast amounts of highly purified RNA.
(C) Frequent misincorporation of unnatural amino acids.
(D) Inability to resolve complex repetitive regions due to short read lengths.

2011

**106.** In structural genome annotation, which of the following signals must bioinformatic algorithms recognize to accurately identify gene boundaries in eukaryotes?
(A) Start codons, stop codons, and splice site donor/acceptor sequences
(B) Poly-A binding proteins and ribosomes
(C) Only the Kozak consensus sequence
(D) EcoRI and HindIII restriction sites

2022

**107.** Forward genetics screens often rely on EMS (Ethyl methanesulfonate) mutagenesis. EMS predominantly induces which specific type of mutation?
(A) Large chromosomal inversions
(B) G/C to A/T transitions
(C) Whole genome duplications
(D) Long terminal repeat retrotransposon insertions

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2014

**36.** Hierarchical Clone-by-Clone sequencing was widely utilized in early large-scale genome projects. What is the initial critical step in this approach?
(A) Direct shearing of the whole genome into 500 bp fragments
(B) Sequencing of millions of short tags simultaneously
(C) Construction of a large-insert BAC library
(D) Application of fluorescent reversible terminators

2008

**37.** In genome annotation, gap filling within scaffolds is frequently achieved by which specialized sequencing or mapping technique?
(A) Optical mapping and long-read sequencing
(B) Northern blotting of total RNA
(C) Two-dimensional gel electrophoresis
(D) Serial Analysis of Gene Expression

2023

**38.** Which specific molecular mechanism does RNA interference (RNAi) exploit to achieve reverse genetic knockdown of target genes?
(A) Mutagenesis via transposon integration into an exon
(B) Deletion of promoter regions via CRISPR-Cas9
(C) Overexpression of the target gene via strong constitutive promoters
(D) Targeted degradation of mRNA guided by homologous dsRNA

2007

**39.** In a dual-color cDNA microarray experiment, a resulting "yellow" signal at a specific spot typically indicates:
(A) Absence of transcript expression in both samples.
(B) Equal abundance of the target transcript in both control and treatment.
(C) High transcript expression exclusively in the reference sample.
(D) A failure of the hybridization protocol.

2002

**40.** Consider the following statements regarding proteomics techniques:
I. MALDI-TOF identifies proteins by measuring the mass-to-charge ratio of ionized peptides.
II. The Yeast Two-Hybrid system is primarily used to detect in vivo protein-protein interactions.
III. 2D-PAGE cannot resolve post-translationally modified proteins.
IV. Trypsin is commonly used to digest proteins into peptides prior to mass spectrometry.
Which of the above statements are correct?
(A) I, II and IV
(B) I and III
(C) II, III and IV
(D) I and II

2015

**41.** In metabolomics, Nuclear Magnetic Resonance (NMR) spectroscopy presents which major advantage over mass spectrometry-based methods?
(A) It has significantly higher sensitivity for trace metabolites.
(B) It requires extensive chemical derivatization prior to analysis.
(C) It relies exclusively on the separation of volatile compounds in a gas phase.
(D) It is non-destructive and allows for complete structural elucidation.

2024

**42.** When profiling plant ionomes using ICP-OES, the fundamental principle of the technique relies on:
(A) The measurement of light emitted by ions excited by high-temperature plasma.
(B) The mass fragmentation patterns of charged molecular ions.
(C) The absorption of specific wavelengths of light by unexcited atoms in a flame.
(D) The radioactive decay of specific isotopes.

2023

**43.** Microsynteny is best defined as the conservation of:
(A) Entire chromosome numbers between a diploid and a tetraploid species.
(B) Gene content and order at the sub-chromosomal level.
(C) Intron lengths across paralogous gene families.
(D) Proteomic expression profiles during a specific developmental stage.

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2017

**44.** During gene pyramiding via Marker-Assisted Selection, what is the breeder's primary objective?
(A) To eliminate all recessive alleles from a population.
(B) To combine multiple resistance genes into a

(C) The fragment will run slower on the agarose gel.
(D) The DNA will spontaneously degrade.

2008

**118.** Which PCR-based marker system typically requires the least amount of prior DNA sequence information for initial implementation?
(A) SSR
(B) CAPS
(C) TaqMan SNP assays
(D) RAPD

2018

**119.** The biochemical basis of AFLP involves the generation of sticky ends. Which enzyme is explicitly responsible for attaching synthetic adapters to these sticky ends?
(A) Taq DNA Polymerase
(B) T4 DNA Ligase
(C) EcoRI Endonuclease
(D) Reverse Transcriptase

2011

**120.** Match the SSR-related concepts in List I with their corresponding descriptions in List II.
List I: SSR-related Concepts
P. Slippage
Q. EST-SSR
R. Flanking region
S. Genomic SSR
List II: Descriptions
1. Derived from transcribed RNA sequences
2. Highly conserved sequence used for primer design
3. Mechanism causing repeat length variation
4. Derived from random non-coding chromosomal sequences
(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-4, R-3, S-2
(C) P-3, Q-4, R-1, S-2
(D) P-2, Q-3, R-4, S-1

2025

**121.** In a diploid genome, the maximum number of alleles a single individual can possess at a specific SNP locus is:
(A) One
(B) Three
(C) Four
(D) Two

2014

**122.** Which derived marker technique amplifies regions situated strictly between adjacent, inversely oriented microsatellite repeats?
(A) Inter-Simple Sequence Repeats (ISSR)
(B) Sequence Characterized Amplified Regions (SCAR)
(C) Cleaved Amplified Polymorphic Sequences (CAPS)
(D) Amplified Fragment Length Polymorphism (AFLP)

2005

**123.** A Recombinant Inbred Line (RIL) population is typically generated from an F2 population through:
(A) Extensive backcrossing to one parent
(B) Successive generations of strict self-pollination
(C) Chromosome doubling using colchicine
(D) Random outcrossing in an isolated block

2014

**124.** The Kosambi mapping function is distinct from the Haldane mapping function because the Kosambi function accounts for:
(A) Incomplete penetrance
(B) Crossover interference
(C) Epistatic interactions
(D) Maternal effects

2021

**125.** The transition from a genetic linkage map (cM) to a physical sequence map (bp) relies heavily on identifying which of the following?
(A) Marker anchors mapped onto sequenced assemblies
(B) Phenotypic correlations
(C) Transcriptional expression variances
(D) Secondary protein structures

2023

**126.** A major limitation of classic Bulked Segregant Analysis (BSA) when mapped via simple RAPD or SSR markers is:
(A) It requires pooling thousands of individuals per bulk.
(B) It cannot be used for disease resistance traits.
(C) It identifies loci within wide chromosomal intervals.
(D) It inherently destroys the extracted DNA.

2017

**127.** Which QTL mapping strategy explicitly incorporates background markers as cofactors to absorb the variance of unlinked QTLs, thereby increasing the detection precision of the target interval?
(A) Simple Interval Mapping (SIM)

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that an individual plant carries three distinct dominant resistance genes if the phenotype only shows generic resistance?
(A) By sequentially exposing the plant to three different temperature stresses.
(B) By strictly measuring the overall height of the plant.
(C) By performing a single unlinked RAPD assay.
(D) Multiplexing three co-dominant markers for each gene.

2017

**183.** Genomic Estimated Breeding Values (GEBVs) represent:
(A) The absolute count of chiasmata occurring during meiosis.
(B) Sum of additive genetic effects from genome-wide markers.
(C) The environmental variance impacting a quantitative trait.
(D) The percentage of rare transversions in an individual.

2023

**184.** In GWAS, the "K-matrix" specifically accounts for which confounding factor in the statistical model?
(A) The physical length of the chromosomes in megabases
(B) The number of coding sequences (exons) present
(C) Familial relatedness among panel individuals.
(D) The frequency of alternative splicing events

2007

**185.** A molecular marker that amplifies multiple bands simultaneously, acting as a random genomic screen across the genome, is defined as a:
(A) Locus-specific marker
(B) Morphological marker
(C) Biochemical marker
(D) Multi-locus marker

2008

**186.** RFLP analysis has largely been replaced by PCR-based systems in modern plant breeding pipelines. Which of the following is NOT a reason for this shift?
(A) PCR inherently provides greater map resolution.
(B) PCR requires vastly smaller amounts of DNA.
(C) PCR-based markers are generally much faster to assay.
(D) PCR avoids the complexities of Southern blotting and probe handling.

2007

**187.** The standard cycling parameters for RAPD amplification utilize a relatively low annealing temperature (e.g., 35°C–40°C). Why is this necessary?
(A) To prevent the Taq polymerase from denaturing.
(B) Short decamers require low temperatures to stably bind.
(C) To actively encourage the formation of single-stranded DNA loops.
(D) Because the target DNA sequences are highly methylated.

2009

**188.** In AFLP, the use of rare cutters (like EcoRI) compared to frequent cutters (like MseI) ensures that:
(A) Optimal fragment sizes and complexity are achieved.
(B) Most generated fragments strictly end in an EcoRI site on both sides.
(C) The genomic DNA is perfectly degraded into single nucleotides.
(D) The adapters cannot ligate to the DNA.

2002

**189.** SSR loci are highly valued for distinguishing closely related cultivars primarily because:
(A) They exhibit hypervariability due to replication slippage.
(B) They strictly mutate via frameshifts that alter visual phenotypes.
(C) They are purely dominant markers, easing visual scoring.
(D) They possess identical sequences across all kingdoms of life.

2017

**190.** Consider the following steps involved in developing high-throughput SNP arrays:
I. Whole-genome resequencing of diverse germplasm
II. Bioinformatics selection of informative SNP panels
III. Synthesis and immobilization of allele-specific oligonucleotides onto a microchip
IV. Hybridization of fluorescently labeled target DNA to the microchip
Which of the following represents the correct sequential arrangement of these steps?
(A) I, II, III, IV

(C) Linkage drag from undesirable flanking alleles
(D) Genomic imprinting

2025

**137.** Which crucial parameter in Genomic Selection evaluates the correlation between the calculated Genomic Estimated Breeding Value (GEBV) and the true breeding value?
(A) Phenotypic variance
(B) Heritability estimate
(C) Prediction accuracy
(D) Kinship coefficient

2001

**138.** In Genome-Wide Association Studies (GWAS), false positives generated by population stratification are statistically mitigated by including which component in the mixed model?
(A) Q-matrix (population structure matrix)
(B) Recombination frequency
(C) LOD scores
(D) Molar mass of DNA

2016

**139.** Which molecular marker system is explicitly categorized as hybridization-based rather than strictly PCR-based?
(A) SCAR
(B) Diversity Arrays Technology (DArT)
(C) SSR
(D) RAPD

2005

**140.** Consider the following statements regarding the principles of RFLP analysis:
I. RFLP analysis inherently detects single nucleotide variations that occur outside of restriction enzyme recognition sites.
II. RFLP utilizes agarose gel electrophoresis to separate digested DNA fragments by size.
III. Southern blotting involves the transfer of DNA to a membrane for probe hybridization.
IV. RFLP markers exhibit co-dominant inheritance, allowing distinction between homozygotes and heterozygotes.
Which of the above statement(s) is/are INCORRECT?
(A) I only
(B) I and IV
(C) II and III
(D) III only

2006

**141.** Why are extremely long primers (e.g., 25-30 bp) generally avoided in RAPD analysis?
(A) They increase primer dimers and reduce the number of potential genomic binding sites.
(B) They denature at temperatures too low for PCR.
(C) They are biologically impossible to synthesize.
(D) They specifically degrade the Taq polymerase.

2013

**142.** The high multiplexing capability of AFLP—often generating 50 to 100 bands per gel lane—is primarily advantageous for:
(A) Direct visual sequencing of individual genes.
(B) Identifying specific single nucleotide transitions.
(C) Rapid genome-wide scanning for polymorphisms.
(D) In vivo visualization of protein expression.

2023

**143.** Which bioinformatic approach is primarily required to develop novel genomic SSR markers for an uncharacterized species?
(A) Western blotting
(B) Flow cytometry of nuclei
(C) Chromosome painting via FISH
(D) Sequencing genomic libraries enriched for repeats

2003

**144.** High-throughput genotyping platforms like KASP do not require gel electrophoresis. How is the SNP variation detected in a KASP assay?
(A) By mass spectrometry
(B) By radioactive autoradiography
(C) By allele-specific fluorescent dye emission
(D) By restriction enzyme fragment visualization

2005

**145.** What is the fundamental difference between a CAPS marker and a dCAPS (derived CAPS) marker?
(A) CAPS utilizes RNA, while dCAPS utilizes DNA.
(B) dCAPS introduces a mismatch to create a restriction site.
(C) CAPS strictly generates dominant data, while dCAPS is strictly co-dominant.
(D) dCAPS requires hybridization with radioactive probes.

2016

**146.** A "Near Isogenic Line" (NIL) developed for a specific disease resistance gene differs from the recurrent parent primarily at:

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(B) RAPD
(C) EST-SSR
(D) TaqMan Assay

2018

**164.** What fundamental limitation dictates that RAPD markers cannot accurately determine heterozygosity in an F2 mapping population?
(A) They are derived from RNA.
(B) They utilize strictly 6-base pair recognition sites.
(C) Dominant inheritance cannot distinguish AA from Aa.
(D) They require radioactive labeling.

2007

**165.** The selective amplification step of AFLP generates polymorphism profiles. How are these amplified fragments typically resolved and visualized in classical protocols?
(A) High-resolution denaturing polyacrylamide gels (PAGE)
(B) Centrifugation on a sucrose gradient
(C) Standard 1% agarose gels stained with ethidium bromide
(D) Simple UV spectrophotometry

2014

**166.** A researcher observes multiple "stutter bands" when amplifying an SSR locus. What molecular phenomenon primarily causes these artifacts during PCR?
(A) Incomplete restriction digestion
(B) Contamination with RNA
(C) Spontaneous transversions during cycling
(D) Taq polymerase slippage across tandem repeats

2022

**167.** Which term specifically describes an SNP substitution where a pyrimidine (e.g., Cytosine) is replaced by a purine (e.g., Adenine)?
(A) Transition
(B) Transversion
(C) Inversion
(D) Translocation

2011

**168.** To convert a polymorphic, dominant RAPD band into a highly specific, co-dominant SCAR marker, the most critical step is:
(A) Radiochemically labeling the original RAPD primer.
(B) Digesting the RAPD fragment with EcoRI.
(C) Designing longer specific primers based on the sequence.
(D) Hybridizing the RAPD fragment to a genomic microarray.

2009

**169.** What is a primary biological limitation of developing a Doubled Haploid (DH) mapping population?
(A) The resulting plants are completely sterile.
(B) Highly genotype-dependent tissue culture requirements.
(C) The individuals are highly heterozygous.
(D) It requires meiotic crossing over to cease.

2008

**170.** Match the linkage mapping parameters/functions in List I with their definitions in List II.
List I: Linkage Mapping Parameters/Functions
P. Recombination Frequency ($\theta$)
Q. LOD Score
R. Kosambi Function
S. Haldane Function
List II: Definitions
1. Assumes no crossover interference
2. Assumes the presence of crossover interference
3. The statistical likelihood logarithm evaluating linkage vs. independent assortment
4. The proportion of non-parental gametes
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-4, Q-3, R-1, S-2
(D) P-1, Q-2, R-3, S-4

NODIA

2024

**171.** If a 100 kb genomic BAC clone corresponds to a 2 cM genetic map distance in a high-recombination region, what is the localized physical-to-genetic ratio?
(A) 50 kb/cM
(B) 100 kb/cM
(C) 200 kb/cM
(D) 500 kb/cM

2017

**172.** When applying BSA to molecular mapping, if a specific SSR marker strictly co-segregates with the "resistant bulk", what banding pattern should be observed?
(A) The marker band will appear equally intense in both bulks.
(B) Marker band appears only in resistant parent and bulk.
(C) The marker band will appear as a smear across all lanes.

(B) RNA-Seq requires prior sequence knowledge to design probes, whereas microarrays do not.
(C) Microarrays strictly analyze protein abundance, whereas RNA-Seq analyzes mRNA.
(D) RNA-Seq cannot detect alternatively spliced variants.

2012

**155.** In protein interaction studies, the Yeast Two-Hybrid system requires the target proteins to interact within which cellular compartment to drive reporter gene expression?
(A) The cytoplasm
(B) The endoplasmic reticulum
(C) The nucleus
(D) The extracellular matrix

2025

**156.** The extraction protocol for metabolomics profiling using GC-MS typically demands the complete removal of which cellular component to prevent analytical interference?
(A) Monosaccharides
(B) Water (samples must be highly desiccated)
(C) Small organic acids
(D) Volatile esters

2023

**157.** Which biological insight is uniquely provided by ionomic profiling using ICP-MS compared to standard transcriptomics?
(A) Direct accumulation and distribution of minerals.
(B) The rate of RNA transcription in root hairs.
(C) The exact physical location of a gene on a chromosome.
(D) The frequency of homologous recombination.

2011

**158.** The concept of "collinearity" in comparative genomics refers strictly to:
(A) The simultaneous expression of genes in the same biochemical pathway.
(B) The merging of two distinct genomes in an allopolyploid.
(C) Conservation of linear gene order along chromosomes.
(D) The physical alignment of DNA and RNA during transcription.

2021

**159.** When executing marker-assisted "foreground selection," the breeder's primary analytical focus is strictly upon:
(A) The percentage of the recurrent parent genome recovered.
(B) The markers located across the background unlinked chromosomes.
(C) The overall phenotypic yield under drought stress.
(D) Precise markers linked to the specific target gene.

2017

**160.** Read the given assertion and reason and select the correct option.
Assertion (A): Genomic Selection (GS) is highly effective for improving complex quantitative traits controlled by many minor QTLs.
Reason (R): GS relies strictly on identifying and introgressing one or two major markers via Marker-Assisted Backcrossing.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001

**161.** In a Genome-Wide Association Study (GWAS), if a population exhibits strong "population structure," failure to account for it statistically will result in:
(A) Perfect mapping resolution.
(B) Genomic Estimated Breeding Values (GEBVs).
(C) Spurious marker-trait associations.
(D) Complete elimination of all significant markers.

2024

**162.** Which of the following features is a distinct advantage of morphological markers over molecular markers?
(A) They are independent of environmental influence.
(B) They exhibit an extremely high degree of polymorphism across genomes.
(C) They allow for the genotyping of dormant seeds.
(D) They are visually scorable in the field without equipment.

2023

**163.** The necessity for high molecular weight, highly pure genomic DNA is a critical bottleneck for which specific marker technique?
(A) RFLP

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(B) Single Marker Analysis (SMA)
(C) Analysis of Variance (ANOVA)
(D) Composite Interval Mapping (CIM)

2015

**128.** In Next-Generation Sequencing, the term "sequencing depth" (or coverage) refers to:
(A) The physical length of the flow cell.
(B) The maximum length of a single continuous read.
(C) Average number of times a nucleotide is sequenced.
(D) The total volume of reagents used.

2024

**129.** During eukaryotic genome annotation, predicting the precise location of exons can be mathematically complex due to:
(A) The absolute lack of stop codons in eukaryotes.
(B) Variable-length introns and alternative splicing.
(C) The circular nature of the nuclear chromosomes.
(D) The continuous translation of the telomeres.

2021

**130.** Consider the following statements regarding functional genomics tools:
I. Transposon tagging inserts known DNA sequences into the genome to disrupt gene function.
II. RNAi requires the introduction of double-stranded RNA homologous to the target gene.
III. T-DNA insertion mutagenesis is completely predictable and targeted to specific chosen loci.
Which of the above statements are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I only

2008

**131.** Serial Analysis of Gene Expression (SAGE) relies on generating concatemers. What is the primary purpose of concatemerization in this protocol?
(A) To sequence multiple short tags simultaneously.
(B) To prevent RNA degradation by RNases.
(C) To strictly hybridize tags to microarrays.
(D) To translate the tags into functional peptides.

2004

**132.** In 2D-PAGE, the first dimension separates proteins through isoelectric focusing. What is the state of a protein when it reaches its isoelectric point (pI)?
(A) Its net electrical charge is highly positive.
(B) Its net electrical charge is highly negative.
(C) Its net electrical charge is zero, stopping migration.
(D) It is completely degraded into constituent amino acids.

2004

**133.** Which physical principle drives the separation of metabolite molecules in the mass analyzer of a typical mass spectrometer?
(A) Ion deflection by fields proportional to $\frac{m}{z}$ ratio
(B) Absorption of infrared light by functional groups
(C) Chromatographic retention time on a non-polar column
(D) Excitation of electrons by radio waves

2013

**134.** Which of the following best explains why ICP-MS is heavily preferred over traditional flame atomic absorption spectroscopy for ionomic studies?
(A) ICP-MS utilizes visible light instead of mass fragments.
(B) ICP-MS does not require sample digestion.
(C) ICP-MS strictly analyzes organic volatile compounds.
(D) High-throughput simultaneous detection of trace isotopes.

2010

**135.** When analyzing the evolution of gene families, paralogous genes typically originate from which mechanism?
(A) Speciation separating a single ancestral population
(B) Whole genome or segmental duplication events
(C) Direct viral transduction across species barriers
(D) Reciprocal chromosomal translocations

NODIA

2025

**136.** In a Marker-Assisted Backcrossing (MABC) program, recombinant selection is performed specifically to minimize which phenomenon?
(A) Epistasis
(B) Pleiotropy

(D) The marker band will strictly correspond to the chloroplast genome.

2016

**173.** What statistical artifact does Composite Interval Mapping (CIM) specifically aim to eliminate by using background marker cofactors?
(A) Detection of "ghost QTLs" from multiple linked QTLs.
(B) RNA degradation
(C) Polymerase slippage
(D) Ascertainment bias in SNPs

2002

**174.** Which sequencing platform is best characterized by generating exceedingly long single-molecule reads (often >10,000 bp), making it ideal for resolving complex repetitive genome assemblies?
(A) Illumina MiSeq
(B) ABI 3730 Sanger Sequencer
(C) Pacific Biosciences (PacBio) SMRT Sequencing
(D) SOLiD system

2023

**175.** During genome annotation, algorithms evaluating sequence homology heavily utilize which foundational bioinformatics tool?
(A) PCR
(B) MALDI-TOF
(C) DArT
(D) BLAST (Basic Local Alignment Search Tool)

2018

**176.** Which of the following best describes the principle of "insertional mutagenesis" as applied in forward genetics?
(A) Utilizing UV light to randomly cause pyrimidine dimers.
(B) Synthesizing specific mRNA sequences to bind genomic DNA.
(C) Disrupting loci with known sequences to tag mutants.
(D) Over-fertilizing plants to induce phenotypic variance.

2019

**177.** An RNA-Seq study yields millions of short sequence reads. What is the fundamental first step in the bioinformatics analysis pipeline to determine transcript expression levels?
(A) Translating the reads directly into proteins
(B) Mapping quality-filtered reads against a reference.
(C) Digesting the reads in silico with restriction enzymes
(D) Running the reads through an NMR simulator

2016

**178.** What primary limitation of 2D-PAGE in proteomics is frequently bypassed by utilizing liquid chromatography-mass spectrometry (LC-MS) shotgun proteomics?
(A) 2D-PAGE requires radioactive labeling.
(B) 2D-PAGE can only separate nucleic acids.
(C) 2D-PAGE strictly denatures all cellular DNA.
(D) Resolution of hydrophobic and extreme-weight proteins.

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2018

**179.** In metabolomics, what is the critical function of a "derivatization" reagent (e.g., MSTFA) prior to GC-MS analysis?
(A) Replacing active hydrogens to increase volatility.
(B) To randomly fragment the metabolites for easier identification.
(C) To strictly separate proteins from the metabolite extract.
(D) To heavily ionize the sample before it enters the gas chromatograph.

2022

**180.** Select the correct logical sequence of operational steps required for standard elemental profiling in ionomics using ICP-MS.
(A) Tissue digestion → Plant tissue sampling → Mass spectrometry → Data analysis
(B) Plant tissue sampling → Acid digestion → Ionization → Detection
(C) Mass spectrometry detection → Plant tissue sampling → Acid digestion → Sample nebulization
(D) Sample nebulization → Acid digestion → Mass spectrometry → Plant tissue sampling

2023

**181.** Which comparative genomics term specifically refers to genes in different species that evolved from a common ancestral gene via a speciation event?
(A) Orthologues
(B) Paralogues
(C) Xenologues
(D) Homoeologues

2022

**182.** During Maker-Assisted Selection for gene pyramiding, how does the breeder confirm

(A) The entire mitochondrial genome.
(B) Exactly 50% of its nuclear alleles.
(C) The chloroplast genome only.
(D) Only the chromosomal segment containing the target.

2019

**147.** Map distance estimation relies heavily on recognizing that double crossovers can occur between two distant markers. What is the mathematical consequence of unobserved double crossovers?
(A) They result in underestimation of true genetic distance.
(B) They result in overestimation of true genetic distance.
(C) They cause the genetic distance to exceed the physical distance.
(D) They convert dominant markers into co-dominant markers.

2015

**148.** Which technique physically isolates intact individual chromosomes to map genes directly onto specific chromosomal bands?
(A) Gas Chromatography
(B) Next-Generation Sequencing
(C) Cytogenetic mapping (e.g., via Flow Karyotyping)
(D) Serial Analysis of Gene Expression

2014

**149.** In BSA, after DNA pooling and molecular marker analysis, a marker that shows equal allele frequencies in both the "resistant bulk" and the "susceptible bulk" is interpreted as:
(A) Genetically unlinked to the resistance trait.
(B) Perfectly linked to the resistance trait.
(C) Epistatically interacting with the resistance trait.
(D) Lethal to the organism.

2016

**150.** Consider the following statements concerning Quantitative Trait Loci (QTL) mapping methodologies:
I. Single Marker Analysis evaluates the association between one marker and the trait variance without calculating map distance.
II. Interval Mapping utilizes pairs of adjacent linked markers to estimate QTL likelihood within the interval.
III. Composite Interval Mapping controls background genetic variance by integrating additional marker cofactors.
IV. Interval Mapping is strictly immune to the effects of ghost QTLs.
V. QTL mapping requires both high-density genotypic data and precise phenotypic evaluation.
Which of the above statements are correct?
(A) I, II, III and V
(B) II, III, IV and V
(C) I, III, IV and V
(D) I, II and IV

2013

**151.** Which major limitation was historically associated with Hierarchical Clone-by-Clone sequencing, prompting the shift towards Whole Genome Shotgun (WGS) sequencing?
(A) Clone-by-clone sequencing strictly required RNA transcripts.
(B) Constructing and mapping BAC libraries was labor-intensive.
(C) It generated sequencing reads that were too short to assemble.
(D) It inherently skipped over gene-coding regions.

2011

**152.** In whole-genome assembly, repetitive DNA sequences (e.g., retrotransposons) cause major bioinformatics challenges, primarily resulting in:
(A) Spontaneous changes to the open reading frames.
(B) Overestimation of the GC content.
(C) Assemblies collapsing into fragmented contigs.
(D) Perfect, gapless chromosomal assemblies.

2015

**153.** A reverse genetics experiment utilizing RNAi targeting a specific lignin-biosynthetic gene results in a plant with weak stems. What does this confirm?
(A) The gene is located strictly on a plasmid.
(B) The RNAi construct directly mutated the genomic DNA sequence.
(C) The plant acquired a novel transposon insertion.
(D) Specific gene transcript is needed for structural integrity.

2004

**154.** What is the fundamental difference between microarray and RNA-Seq in transcriptomic studies?
(A) Microarray uses predefined probes; RNA-Seq sequences all.

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(B) II, I, III, IV
(C) III, II, I, IV
(D) II, I, IV, III

2010

**191.** The Inter-Simple Sequence Repeat (ISSR) technique utilizes a single primer. How does this primer differ fundamentally from a standard RAPD primer?
(A) It is perfectly random and strictly 10 bases long.
(B) It is composed entirely of RNA nucleotides.
(C) It is an exact copy of the T-DNA left border.
(D) It uses a specific repeat sequence with short anchors.

2003

**192.** Which genetic phenomenon allows a Recombinant Inbred Line (RIL) population to offer a higher mapping resolution than an F2 population of identical size?
(A) RILs possess strictly unlinked centromeres.
(B) RILs mutate at an exponentially higher rate than F2s.
(C) Successive selfing accumulates meiotic recombinations.
(D) RILs undergo spontaneous whole genome duplications.

2013

**193.** The calculation of LOD scores in linkage analysis relies upon taking the base-10 logarithm of the ratio of two likelihoods. What are these two likelihoods?
(A) The likelihood of survival vs. the likelihood of mortality
(B) Linked at specific fraction vs. independent assortment.
(C) The likelihood of a dominant allele vs. a recessive allele
(D) The likelihood of gene silencing vs. active expression

2004

**194.** Which of the following scenarios best explains a severe discrepancy where two markers are mapped 5 cM apart genetically, but physically separated by 10 Megabases (Mb) of sequence?
(A) The markers are located in a highly recombinogenic euchromatic region.
(B) The species undergoes strictly asexual reproduction.
(C) The markers are unlinked and reside on different chromosomes.
(D) Markers are in pericentromeric regions with low crossover.

2019

**195.** In applying BSA, the "susceptible bulk" DNA profile shows bands from both Parent A (resistant) and Parent B (susceptible) at a target locus, whereas the "resistant bulk" only shows Parent A's band. This implies the resistance trait is most likely:
(A) Dominant
(B) Recessive
(C) Lethal
(D) Epigenetic

2010

**196.** Simple Interval Mapping (SIM) assumes which specific configuration of QTLs within the mapping interval?
(A) Infinite polygenic QTLs
(B) Strict epistasis between two tightly linked QTLs
(C) Exactly one QTL between two flanking markers.
(D) No QTLs whatsoever

2007

**197.** Which inherent challenge of Whole Genome Shotgun (WGS) sequencing is explicitly overcome by standard paired-end (or mate-pair) sequencing libraries?
(A) Spanning repetitive elements with distance constraints.
(B) The high error rate of DNA polymerases
(C) Resolving the absolute size of the restriction fragments
(D) The degradation of RNA templates

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2006

**198.** In automated genome annotation pipelines, what is the primary role of ab initio gene prediction algorithms?
(A) Identifying gene structures strictly by aligning them to known RNA transcripts from databases.
(B) Digesting the DNA with restriction endonucleases.
(C) Visualizing the chromosomes under a light microscope.
(D) Predicting genes based entirely on statistical properties.

2009

**199.** A reverse genetics methodology utilizing CRISPR-Cas9 fundamentally differs from standard EMS mutagenesis by offering:
(A) Non-heritable trait modifications exclusively.

(B) Precise, targeted modification of a predefined sequence.
(C) Strictly dominant lethal mutations.
(D) The mandatory insertion of viral DNA.

2022

**200.** Read the given assertion and reason and select the correct option.
Assertion (A): Microarray analysis is highly dependent upon prior knowledge of the target genome's sequence.
Reason (R): Probes immobilized on the microarray chip must be pre-synthesized to physically match known transcript sequences.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**201.** Which ionization technique used in mass spectrometry acts "softly," allowing for the analysis of large, intact protein molecules without extensive fragmentation?
(A) Inductively Coupled Plasma (ICP)
(B) Matrix-Assisted Laser Desorption/Ionization
(C) Electron Impact (EI) ionization
(D) Thermal combustion methods

2024

**202.** What distinct analytical advantage does Liquid Chromatography (LC) possess over Gas Chromatography (GC) in metabolomics?
(A) LC analyzes non-volatile compounds without derivatization.
(B) LC perfectly separates volatile gaseous mixtures.
(C) LC inherently requires significantly higher operating temperatures than GC.
(D) LC operates strictly in a vacuum.

2014

**203.** Which essential physiological insight can be robustly derived by integrating ionomic data with transcriptomic data under heavy metal stress?
(A) The exact physical structure of the heavy metal atom
(B) Identifying transporters correlated with accumulation.
(C) The precise sequence of the centromeric satellite repeats
(D) The visual color of the plant's flowers

2015

**204.** "Orthologues" and "Paralogues" fundamentally differ based on their evolutionary origin. Which event exclusively defines the divergence of paralogous genes?
(A) Speciation events
(B) Gene duplication events
(C) Horizontal gene transfer
(D) Reverse transcription events

2024

**205.** In a standard MAS backcrossing scheme targeting a single recessive resistance gene, why must the breeder strictly self-pollinate the final backcross generation?
(A) To fix target genes in a homozygous recessive state.
(B) To induce random mutations in the background genome.
(C) To physically remove the dominant susceptible allele from the cell.
(D) To evaluate the rate of LD decay.

2007

**206.** Genomic Selection (GS) prediction models like Ridge Regression BLUP (RR-BLUP) enforce a statistical "shrinkage" penalty. What mathematical problem does this primarily address?
(A) Having zero marker variations in the target population
(B) The excessive physical size of the genomic chromosomes
(C) The $p > n$ problem where markers exceed observations
(D) The structural degradation of DNA molecules over time

2003

**207.** A major statistical consequence of Linkage Disequilibrium (LD) rapidly decaying within very short physical distances (e.g., in outcrossing species like maize) is that GWAS requires:
(A) A drastically reduced number of markers.
(B) Exclusively dominant markers.
(C) Strictly mapping purely qualitative traits.
(D) Extremely high density of genome-wide markers.

2023

**208.** Which class of molecular markers relies heavily on identifying variations strictly inherited through the maternal lineage in most agronomic crops?
(A) Nuclear SSRs
(B) RFLPs located on autosomes

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(C) Chloroplast specific markers
(D) Y-chromosome specific markers

2012

**209.** During RFLP analysis, incomplete restriction digestion by the endonuclease most frequently leads to:
(A) The conversion of dominant markers into co-dominant markers.
(B) Appearance of smears and false-positive extra bands.
(C) The spontaneous synthesis of novel DNA.
(D) Perfect single-band resolution across all lanes.

2025

**210.** Match the genome analysis techniques in List I with their primary applications in List II.
List I: Genome Analysis Techniques
P. RNA-Seq
Q. SAGE
R. 2D-PAGE
S. MALDI-TOF
List II: Primary Applications
1. Identification of peptide mass fingerprints
2. High-throughput separation of intact proteins
3. De novo transcript discovery and quantification via sequencing
4. Transcript quantification via short concatemerized tags
(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-3, Q-2, R-4, S-1
(D) P-1, Q-4, R-2, S-3

2016

**211.** Which PCR optimization strategy is most universally effective in reducing the non-specific amplification background in an AFLP reaction?
(A) Implementing a "touch-down" PCR cycling profile.
(B) Using exclusively random 5-mer primers
(C) Decreasing the concentration of Taq polymerase to zero
(D) Eliminating magnesium chloride from the reaction buffer

2011

**212.** If a specific SSR locus amplifies a 150 bp fragment in Parent A and a 160 bp fragment in Parent B, what fragment profile is theoretically expected in their strictly heterozygous $F_1$ progeny?
(A) A single 155 bp band
(B) A single 310 bp band
(C) Both the 150 bp and 160 bp bands
(D) Neither band

2022

**213.** TaqMan assays utilize a dual-labeled probe containing a fluorophore and a quencher. Under what condition is the fluorescent signal actively emitted?
(A) Exonuclease activity cleaves probe and separates dye.
(B) When the probe perfectly hybridizes but remains fully intact
(C) When the restriction enzyme digests the template DNA
(D) When the RNA is translated into a fluorescent protein

2019

**214.** Sequence Characterized Amplified Region (SCAR) markers inherently offer which major advantage over their parent RAPD markers?
(A) They randomly scan the entire genome.
(B) They are exclusively inherited via chloroplasts.
(C) They require completely unpurified DNA.
(D) High robustness and target-specific amplification.

2010

**215.** A mapping population developed by fertilizing an egg with irradiated pollen followed by embryo rescue and chromosomal doubling using colchicine yields:
(A) F2 bulk segregants
(B) Doubled Haploids (DH)
(C) Recombinant Inbred Lines (RILs)
(D) Backcross derivatives

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2004

**216.** Two distinct genetic markers demonstrate a recombination fraction of 0.05. According to the simplest map distance estimate (ignoring mapping functions), they are positioned approximately:
(A) 0.05 cM apart
(B) 5.0 cM apart
(C) 50.0 cM apart
(D) 500.0 cM apart

2012

**217.** When comparing genetic and physical maps, which specific biological feature causes physical distances to appear heavily compressed on a genetic map?
(A) Highly recombinogenic telomeres
(B) Active transcriptional promoters

# ANSWERS

**1.** (B) Co-dominant markers can distinguish heterozygous individuals from homozygous dominant individuals.
Co-dominant molecular markers provide a distinct advantage in genetic analysis by identifying specific allelic combinations at a locus. Unlike dominant markers, which only indicate the presence or absence of a band, co-dominant systems allow for the clear differentiation of heterozygous individuals from homozygous dominant ones. This characteristic is essential for accurate linkage mapping and population studies in diploid organisms.

**2.** (D) To transfer restriction-digested DNA fragments from an agarose gel to a solid membrane.
Southern blotting is a fundamental technique used to immobilize DNA fragments for further analysis during the RFLP workflow. After DNA is digested and separated by size via gel electrophoresis, the fragments are transferred from the fragile agarose gel onto a stable solid membrane, such as nitrocellulose or nylon. This ensures they remain in their relative positions for probe hybridization.

**3.** (A) 10 nucleotides
Randomly Amplified Polymorphic DNA assays rely on the use of short, arbitrary primers to scan the genome for variations. These primers are typically ten nucleotides in length, providing enough specificity to bind at multiple random locations under low-stringency conditions. This length is optimized to ensure a sufficient number of discrete amplification products for distinguishing different genotypes effectively without prior sequence knowledge.

**4.** (C) To generate fragments of optimal size for amplification and to reduce the complexity of the restriction digest.
The combination of a rare-cutting enzyme and a frequent-cutting enzyme is a strategic design in the AFLP protocol. This approach ensures that the majority of the resulting DNA fragments fall within a size range suitable for efficient PCR amplification and high-resolution gel electrophoresis. Furthermore, it significantly reduces the overall complexity of the mixture, allowing for clear and reproducible visualization.

**5.** (A) Slipped-strand mispairing during replication
Simple Sequence Repeats exhibit high levels of allelic variation primarily due to a mutational process known as slipped-strand mispairing. During DNA replication, the polymerase may detach and reattach incorrectly within these repetitive regions. This error leads to the insertion or deletion of repeat units, resulting in the length polymorphisms that make microsatellites highly informative markers for genetic mapping and diversity.

**6.** (D) Transition mutation
Single Nucleotide Polymorphisms are classified based on the nature of the chemical substitution involved in the mutation. A change from Adenine to Guanine represents a substitution between two purines, which is specifically categorized as a transition mutation. These mutations occur more frequently in the genome than transversions, which involve swapping a purine for a pyrimidine, and are crucial in genomics.

**7.** (B) Restriction endonuclease digestion of the PCR product
Cleaved Amplified Polymorphic Sequences markers are developed by combining the specificity of PCR with the sensitivity of restriction endonucleases. After amplifying a specific target region, the resulting PCR product is subjected to digestion by a chosen restriction enzyme. Variations in the DNA sequence at the recognition site will lead to different fragment patterns when visualized on an electrophoresis gel.

**8.** (C) Recombinant Inbred Lines (RILs)
Recombinant Inbred Lines require several generations of systematic selfing to reach a state of near-complete homozygosity. This intensive process makes them more time-consuming to develop compared to other mapping populations like F2 or backcrosses. Because they are genetically fixed, RILs represent a permanent resource for multi-environment phenotypic evaluations and

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(B) RNA-seq
(C) Bisulfite sequencing
(D) CRISPR-Cas9

2023

**228.** In MAS background selection, what is the theoretical expected proportion of the recurrent parent genome recovered in a $BC1F_1$ individual?
(A) 25%
(B) 50%
(C) 75%
(D) 93.75%

2006

**229.** Genomic Selection utilizes a "training population" to establish prediction models. Once the model is highly accurate, it is applied to a "breeding population" which requires:
(A) Only genotypic data to predict breeding values.
(B) Strict phenotypic field trials for all individuals.
(C) Complete ignorance of the training population's data.
(D) The physical elimination of all dominant markers.

2004

**230.** Consider the following analytical steps in executing a standard Genome-Wide Association Study (GWAS):
I. Assembling a diverse germplasm panel and recording phenotypic trait data
II. Genotyping the association panel with high-density markers
III. Controlling for population structure and kinship in a mixed linear model
IV. Identifying statistically significant marker-trait associations based on p-value thresholds
Which of the following represents the correct chronological sequence of these steps?
(A) I, II, III, IV
(B) II, I, III, IV
(C) III, I, II, IV
(D) I, III, II, IV

2004

**231.** Which biochemical property ensures that PCR-based markers (like SSRs) demand vastly lower quantities of starting template DNA than hybridization-based markers (like RFLPs)?
(A) The direct binding of radioactive phosphorus to the template
(B) Geometric amplification of targets by polymerase.
(C) The highly specific cleavage action of endonucleases
(D) The natural degradation of unamplified DNA

2023

**232.** If a specific RFLP probe hybridizes strictly to a single-copy sequence located within a gene that exists only once per haploid genome, how many bands are generally expected on a Southern blot of a homozygous diploid individual?
(A) None
(B) Two
(C) One
(D) A continuous smear

2012

**233.** The inability of RAPD primers to distinguish between homozygous dominant and heterozygous states fundamentally restricts their use in evaluating which genetic parameter?
(A) The number of chromosomes
(B) The rate of photosynthesis
(C) Chloroplast genome size
(D) Allele frequencies and Hardy-Weinberg statistics.

2007

**234.** In the standard AFLP protocol, why must the restriction endonucleases be completely heat-inactivated before the selective amplification step?
(A) To prevent re-cleavage of amplified fragments.
(B) To prevent the random degradation of the Taq polymerase
(C) To strictly synthesize RNA molecules
(D) To ensure the adapters spontaneously fall off

2025

**235.** Which genomic feature ensures that Expressed Sequence Tag-Simple Sequence Repeats (EST-SSRs) exhibit higher transferability across related species than standard genomic SSRs?
(A) EST-SSRs strictly originate from hypervariable telomeric repeats.
(B) Location within highly conserved coding sequences.
(C) EST-SSRs are completely devoid of any mutational constraints.
(D) EST-SSRs strictly lack any flanking regions.

2010

**236.** High-throughput SNP genotyping via microarray technology typically relies heavily upon which specific molecular property?

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isoelectric focusing. In the second dimension, they are separated according to their molecular weight using SDS-PAGE. This dual-step process allows for the visualization of thousands of distinct protein spots.

**18.** (D) Volatile and thermally stable metabolites
Gas Chromatography-Mass Spectrometry is a highly sensitive analytical technique ideally suited for the identification and quantification of small molecules in metabolomics. It requires that the target compounds be volatile or capable of being made volatile through chemical derivatization. Because the separation occurs in a gas phase at elevated temperatures, the metabolites must also remain thermally stable during the analysis.

**19.** (A) Sensitive, simultaneous quantification of multiple isotopes.
Inductively Coupled Plasma Mass Spectrometry is a cornerstone technology in ionomics due to its exceptional sensitivity and broad dynamic range. It allows for the simultaneous detection and quantification of a wide array of elements and their isotopes within a biological sample. By ionizing the sample in a high-temperature plasma, it provides precise elemental profiles essential for studying mineral nutrient homeostasis.

**20.** (A) P-3, Q-4, R-1, S-2
Understanding comparative genomics requires distinguishing between different types of homologous genes based on their evolutionary history and genomic organization.
**Genomic Terminology and Definitions:**
P. Orthologues arise through speciation events.
Q. Paralogues result from gene duplication within a species.
R. Xenologues involve horizontal transfer between organisms.
S. Macrosynteny describes large-scale gene order conservation.

**21.** (A) Accelerating the recovery of the recurrent parent genome across non-target chromosomes.
Background selection is a strategic component of marker-assisted backcrossing aimed at maximizing breeding efficiency. While foreground selection focuses on the target gene, background selection utilizes markers distributed across the entire genome to identify individuals that have recovered the greatest proportion of the recurrent parent's genetic material. This process significantly reduces the number of backcross generations required to develop near-isogenic lines.

**22.** (D) RR-BLUP assuming equal marker variances
Ridge Regression Best Linear Unbiased Prediction is a widely used statistical model in genomic selection for predicting breeding values. The model operates under the fundamental assumption that every molecular marker across the genome contributes to the trait. Specifically, it assumes that the effects of all markers are drawn from a single normal distribution, each having an equal contribution to the variance.

**23.** (B) Population structure and stratification
In Genome-Wide Association Studies, the presence of population structure can lead to spurious associations between markers and traits. Incorporating a Q-matrix into the statistical model allows researchers to account for the underlying stratification and subgroups within the study population. This correction is vital for reducing false positives and ensuring that the identified associations are truly due to genetic linkage.

**24.** (C) ISSR
Inter-Simple Sequence Repeat markers are generated through the PCR amplification of genomic regions located between two closely spaced, oppositely oriented microsatellite repeats. This technique utilizes a single primer composed of a repeat sequence, often with a short anchoring sequence at one end. ISSR markers are highly polymorphic and provide a useful tool for genetic diversity studies without requiring prior sequence information.

**25.** (D) Southern blotting
Southern blotting is the specific procedural step in RFLP analysis where DNA fragments are moved from an electrophoresis gel to a solid support. This transfer is typically achieved through capillary action or electrophoretic forces, ensuring that the spatial arrangement of the fragments is maintained on the membrane. Once immobilized, the DNA is ready for hybridization with specific labeled probes for visualization.

**26.** (A) They are sensitive to minor PCR variations, causing poor reproducibility.
RAPD markers are often criticized in large-scale collaborative projects due to significant challenges with laboratory reproducibility. Because the

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mRNA from the overwhelming abundance of ribosomal RNA (rRNA)?
(A) The 5' methylguanosine cap
(B) The presence of spliced introns
(C) The double-stranded secondary structures
(D) The 3' poly-A tail

2018

**247.** The primary reason a proteomics researcher would apply trypsin digestion prior to mass spectrometry analysis (a "bottom-up" proteomics approach) is to:
(A) To denature the target proteins completely before analysis
(B) To generate predictable peptides for better mass spectrometry
(C) To directly sequence the mRNA transcripts within the sample
(D) To induce specific post-translational phosphorylation events

2004

**248.** In Nuclear Magnetic Resonance (NMR) spectroscopy applied to metabolomics, the specific "chemical shift" (measured in ppm) of a hydrogen atom provides critical information regarding:
(A) The absolute mass of the entire molecule.
(B) The speed of the molecule within a vacuum tube.
(C) Local electronic and chemical environments.
(D) The total number of carbon atoms in the molecule.

2016

**249.** Which elemental interaction concept is critical in ionomics when interpreting data showing that extreme zinc (Zn) deficiency simultaneously causes massive iron (Fe) hyperaccumulation in roots?
(A) Independent assortment of minerals
(B) Strictly random diffusion
(C) Isotopic radioactive decay
(D) Elemental crosstalk or interaction networks

2016

**250.** Conserved collinearity (macrosynteny) across grass genomes (e.g., rice, wheat, and maize) famously allows researchers to:
(A) Predict gene locations based on model genomes.
(B) Assume all grass species possess exactly the same number of chromosomes.
(C) Completely bypass the need for any physical gene mapping.
(D) Prove that horizontal gene transfer strictly dominates grass evolution.

**********

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**35.** (A) It confounds QTL magnitude with recombination frequency.
Single Marker Analysis is the simplest statistical approach for QTL mapping, but it has a major inherent limitation. It cannot distinguish between the effect size of a QTL and the genetic distance between the marker and the QTL. A large-effect QTL that is loosely linked to a marker may produce the same statistical signal as a small-effect QTL tightly linked.

**36.** (C) Construction of a large-insert BAC library
Hierarchical Clone-by-Clone sequencing is a systematic strategy used to assemble large and complex genomes. The initial critical step involves creating a library of large DNA inserts, typically using Bacterial Artificial Chromosomes. These clones are then organized into a physical map based on their overlaps. This structured approach allows the genome to be subdivided into manageable pieces for subsequent sequencing and assembly.

**37.** (A) Optical mapping and long-read sequencing
Closing the gaps within genomic scaffolds is a challenging task in genome assembly, often requiring specialized techniques. Optical mapping provides a high-resolution physical map of the genome, while long-read sequencing technologies can span complex repetitive regions that are difficult for short-read platforms to resolve. Together, these methods allow researchers to bridge remaining gaps and produce a more complete and continuous genome.

**38.** (D) Targeted degradation of mRNA guided by homologous dsRNA
RNA interference is a powerful reverse genetics tool used to study gene function by reducing the expression of specific targets. The process is initiated by the introduction of double-stranded RNA molecules that are homologous to the target gene sequence. These dsRNAs guide a cellular protein complex to recognize and degrade the corresponding messenger RNA, effectively knocking down the gene's activity.

**39.** (B) Equal abundance of the target transcript in both control and treatment.
In a dual-color cDNA microarray experiment, two different samples are labeled with distinct fluorescent dyes, typically red and green. When the samples are hybridized to the same spot on the chip, the resulting color indicates the relative abundance of the transcript. A yellow signal occurs when both the red and green dyes are present in equal intensities, representing equivalent expression levels.

**40.** (A) I, II and IV
Proteomics involves a variety of techniques designed to characterize the full complement of proteins in a biological system.
**Evaluation of Proteomics Methods:**
I. MALDI-TOF identifies peptides based on $\frac{m}{z}$ ratios.
II. Yeast Two-Hybrid detects in vivo protein interactions.
III. 2D-PAGE effectively resolves post-translationally modified proteins.
IV. Trypsin is the standard enzyme for protein digestion.

**41.** (D) It is non-destructive and allows for complete structural elucidation.
Nuclear Magnetic Resonance spectroscopy is a highly valuable tool in metabolomics due to its unique analytical properties. Unlike mass spectrometry, NMR is non-destructive, meaning the sample can be recovered after analysis. Furthermore, it provides detailed information about the chemical environment of atoms, allowing for the complete and unambiguous determination of the three-dimensional structures of diverse metabolic compounds.

**42.** (A) The measurement of light emitted by ions excited by high-temperature plasma.
Inductively Coupled Plasma Optical Emission Spectroscopy is a technique used for the sensitive detection of elements within a sample. It operates by introducing the sample into an extremely high-temperature argon plasma, which vaporizes and excites the constituent atoms and ions. As these excited species return to a lower energy state, they emit light at characteristic wavelengths, which is then quantified.

**43.** (B) Gene content and order at the sub-chromosomal level.
Microsynteny refers to the conservation of gene sequences and their relative organization within small, localized regions of the genome across different species. While macrosynteny describes large-scale chromosomal similarities, microsynteny focuses on finer details at the level of individual genes and small gene clusters. Studying these patterns provides deep insights

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high-resolution mapping projects across different years and locations.

**9.** (D) Haldane mapping function
The Haldane mapping function is a mathematical model used to convert recombination frequencies into genetic map distances. It operates under the specific assumption that crossovers occur randomly and independently along the chromosome, meaning there is no crossover interference. This model accounts for the possibility of double crossovers, which are otherwise undetectable between two widely spaced genetic markers during linkage analysis.

**10.** (D) A is false but R is true.
The physical distance across a chromosome is not strictly proportional to its genetic distance because recombination is not uniformly distributed throughout the DNA. While physical length is measured in base pairs, genetic distance depends on crossover frequencies. Recombination is often suppressed in heterochromatic regions like centromeres, meaning the ratio between these two measures varies significantly between different chromosomal segments.

**11.** (B) Contrasting phenotypic expression of the target trait
Bulked Segregant Analysis is a specialized technique used to rapidly identify molecular markers linked to specific genes of interest. The methodology involves creating two distinct DNA pools from individuals in a segregating population. These pools are constructed by selecting plants that show extreme, contrasting phenotypes for the target trait, such as high resistance versus high susceptibility to a specific disease.

**12.** (B) Additional markers as covariates to control background genetic effects
Composite Interval Mapping provides a significant statistical improvement over simple methods by incorporating multiple markers into the analysis. By using additional markers as covariates, the model effectively controls for the background genetic effects of other QTLs elsewhere in the genome. This approach reduces residual variance, minimizes the detection of false-positive QTLs, and increases the precision of locating the target locus.

**13.** (A) 454 Life Sciences (Roche)
Pyrosequencing is a DNA sequencing technology based on the principle of detecting the release of pyrophosphate during nucleotide incorporation. The 454 Life Sciences platform historically utilized this chemistry to generate sequence data through light emission triggered by enzymatic reactions. While it offered longer reads than other early high-throughput systems, it has largely been superseded by newer technologies with higher output.

**14.** (C) To order and orient contigs using paired-end information.
During the process of genome assembly, scaffolding serves as a critical step in organizing the initial sequence data into a coherent structure. It uses information from paired-end or mate-pair reads to determine the relative order and orientation of individual contigs. By bridging the gaps between fragments with known distances, scaffolding allows for the reconstruction of longer, more continuous chromosomal segments.

**15.** (D) From mutant phenotype to identification of the causative gene
Forward genetics is a classical approach used to determine the genetic basis of a specific biological trait. The workflow begins with the identification of an interesting mutant phenotype, often generated through random mutagenesis or found in natural populations. Researchers then utilize mapping and sequencing techniques to move from this observed trait toward the discovery and characterization of the underlying gene.

**16.** (C) The generation of short sequence tags from the 3' ends.
Serial Analysis of Gene Expression is a technique used to quantify the abundance of various mRNA transcripts within a biological sample. It functions by capturing short, unique sequence tags from the 3' ends of the transcripts. These tags are then concatenated, cloned, and sequenced, allowing researchers to determine the relative expression levels of thousands of genes simultaneously through digital counting.

**17.** (B) Isoelectric point (pI) and molecular weight
Two-dimensional polyacrylamide gel electrophoresis is a powerful method for the high-resolution separation of complex protein mixtures. In the first dimension, proteins are separated based on their isoelectric point through

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(A) The ability of specific restriction enzymes to strictly cut RNA
(B) Single-base mismatch discrimination on a surface.
(C) The physical migration speed of the whole chromosome in an electrical field
(D) The exact tertiary folding structure of the target DNA

2005

**237.** The fundamental design of a Cleaved Amplified Polymorphic Sequence (CAPS) marker integrates the high target specificity of PCR with the localized sequence interrogation of:
(A) Restriction endonucleases
(B) Polyacrylamide gels
(C) RNA polymerases
(D) Mass spectrometers

2001

**238.** In developing a Near Isogenic Line (NIL), after numerous generations of backcrossing, the targeted introgressed segment often remains flanked by a small segment of the donor parent's genome. This phenomenon is termed:
(A) Independent assortment
(B) Genomic imprinting
(C) Linkage drag
(D) Centromeric suppression

2014

**239.** The mathematical formulation $X = -0.5\ln(1-2\theta)$ precisely defines which linkage mapping function?
(A) The Lander-Green function
(B) The Kosambi function
(C) The Morgan function
(D) The Haldane function

2018

**240.** The conversion parameter mapping genetic distance (cM) to physical distance (bp/Mb) is defined as:
(A) The mutation rate
(B) The LOD score
(C) The mapping resolution or cM/Mb ratio
(D) The heritability index

2022

**241.** A major advantage of executing Bulked Segregant Analysis (BSA) via Next-Generation Sequencing (QTL-seq) rather than traditional markers is:
(A) It strictly limits the analysis to one specific known gene.
(B) Simultaneous mapping and massive genome-wide SNP data.
(C) It inherently requires zero computational analysis.
(D) It completely bypasses the need to identify phenotypic extremes.

2016

**242.** In QTL mapping via Interval Mapping (IM), the "likelihood ratio" peaks exactly at the statistical center of the mapping interval. This implies:
(A) The QTL is situated perfectly on top of one flanking marker.
(B) The interval contains zero QTLs.
(C) The trait is purely qualitative.
(D) Highest QTL probability is located between markers.

2005

**243.** The primary rationale for developing "Hierarchical Clone-by-Clone" sequencing protocols during the early human and plant genome projects was to:
(A) Subdivide complex genomes into ordered fragments.
(B) Prevent the need for any bioinformatics software.
(C) Strictly analyze only the expressed mRNA.
(D) Guarantee an absolute zero error rate in raw base calling.

2001

**244.** In genome annotation, sequences homologous to known genes but containing debilitating mutations preventing functional expression are properly annotated as:
(A) Exons
(B) Promoters
(C) Pseudogenes
(D) Origin of replication sites

2007

**245.** A researcher utilizing a transposon-tagging strategy in maize identifies a striking albino mutant phenotype. The most direct subsequent step to identify the causative mutated gene is to:
(A) Sequencing flanking DNA using transposon primers.
(B) Visually inspect the chloroplasts under a light microscope.
(C) Expose the plant to heavy metal stress.
(D) Cross the plant with a wild-type line and strictly measure yield.

2017

**246.** Which structural feature of eukaryotic mRNA is explicitly exploited by RNA-Seq protocols (utilizing oligo-dT beads) to selectively isolate

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technique uses short primers and low annealing temperatures, the resulting banding patterns are highly sensitive to minor fluctuations in PCR conditions, such as reagent concentrations or thermal cycler performance. This sensitivity makes it difficult to compare results between different research facilities accurately.

**27.** (B) They contain 1 to 3 additional selective nucleotides at their 3' ends.
The selective amplification step in the AFLP protocol is designed to reduce the complexity of the DNA fragment mixture. This is achieved by using primers that are identical to the pre-amplification primers but feature one to three additional nucleotides at their 3' ends. Only fragments that perfectly match these selective extensions will be amplified, allowing for clear visualization of discrete bands.

**28.** (B) EST-SSRs are situated in highly conserved coding regions.
Simple Sequence Repeats derived from Expressed Sequence Tags typically exhibit lower levels of polymorphism than those found in non-coding genomic regions. This reduced variation occurs because EST-SSRs are located within or near functional genes, which are subject to stronger evolutionary constraints and purifying selection. Any significant length changes in these regions could disrupt gene function, leading to lower observed diversity.

**29.** (A) Competitive binding of two allele-specific forward primers and a reverse primer
Kompetitive Allele Specific PCR is a high-throughput genotyping technology designed for the precise detection of Single Nucleotide Polymorphisms. The assay utilizes two different forward primers, each specific to one of the alleles, and a common reverse primer. The competition between these primers during the PCR reaction, combined with a fluorescence-based detection system, allows for the clear identification of individual genotypes.

**30.** (B) III only
Different molecular markers have varying requirements for prior sequence knowledge. SCAR and CAPS markers both require specific sequences to design primers or identify restriction sites.
**Analysis of Statements:**

I. SCAR markers are derived from sequenced RAPD fragments.
II. CAPS markers require sequence data for restriction sites.
III. ISSR primers are based on repeat motifs and do not require prior genome sequencing.

**31.** (D) Recombinant Inbred Lines (RILs)
Recombinant Inbred Lines are considered an immortal mapping population because they have achieved a state of nearly complete homozygosity through repeated selfing. Once established, these lines can be propagated by seed indefinitely without further genetic segregation. This stability allows the same genetic material to be shared and evaluated across multiple environments and research trials, making them an invaluable genetic resource.

**32.** (C) 3.0
The LOD score is a statistical measure used in genetic mapping to evaluate the likelihood that two loci are linked rather than assorting independently. A threshold of 3.0 is standardly accepted in the scientific community, representing a 1000-to-1 ratio in favor of linkage. Reaching this value provides sufficient statistical confidence to conclude that the markers or genes are physically connected.

**33.** (B) Pericentromeric heterochromatin
The relationship between genetic map distance and physical chromosomal length varies significantly across different regions of the genome. In pericentromeric heterochromatin, the frequency of meiotic crossing-over is heavily suppressed due to the dense packing of DNA and the presence of specialized proteins. Consequently, these regions show a very low ratio of centimorgans to megabases, appearing compressed on a genetic map.

**34.** (B) 10 to 20 individuals
In Bulked Segregant Analysis, the size of the DNA pools is a critical factor for maintaining sensitivity. Pooling approximately 10 to 20 individuals per bulk is typically recommended to ensure that the unique allelic contributions of each individual are represented. Using too many individuals can dilute the signal of rare alleles, making it difficult to identify markers tightly linked to the trait.

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(C) Exon-dense euchromatic arms
(D) Pericentromeric regions with low crossover rates.

2007

**218.** The fundamental statistical premise of Bulked Segregant Analysis (BSA) relies on the expectation that markers randomly dispersed across the genome (unlinked to the trait) will exhibit what behavior in the two phenotypic bulks?
(A) Equal allelic representation across both bulks.
(B) They will strongly co-segregate with the resistance phenotype.
(C) They will appear strictly homozygous in both bulks.
(D) They will completely fail to amplify.

2004

**219.** Which algorithmic improvement in QTL mapping specifically accounts for the effects of multiple intervening QTLs by using multiple regression and marker cofactors?
(A) Simple Interval Mapping (SIM)
(B) Composite Interval Mapping (CIM)
(C) Single Marker Analysis (SMA)
(D) Non-parametric correlation

2024

**220.** Select the correct sequential order of operational steps for generating a standard Next-Generation Sequencing (NGS) library.
(A) Adapter ligation → DNA fragmentation → PCR amplification → Sequencing
(B) DNA fragmentation → Adapter ligation → PCR → Sequencing
(C) PCR amplification → DNA fragmentation → Sequencing → Adapter ligation
(D) Sequencing → PCR amplification → Adapter ligation → DNA fragmentation

2016

**221.** In genome assembly, what specialized class of sequencing reads is strictly necessary to orient assembled contigs into larger scaffolds across un-sequenced gaps?
(A) Paired-end reads with known insert distances.
(B) Short, single-end 50 bp reads
(C) Expressed Sequence Tags (ESTs)
(D) Highly degraded ancient DNA reads

2024

**222.** An investigator applies EMS to a wild-type seed batch, plants them, and visually screens the resulting M2 generation to discover plants with abnormal root gravitropism. This methodology strictly defines:
(A) Reverse Genetics
(B) Comparative Genomics
(C) Forward Genetics
(D) Transcriptomics

2005

**223.** Identify the principal limitation of serial analysis of gene expression (SAGE) compared to modern high-throughput RNA-Seq.
(A) SAGE cannot be automated.
(B) SAGE fundamentally destroys all cellular DNA.
(C) SAGE only works on prokaryotic organisms.
(D) Short tags may map ambiguously to gene families.

2013

**224.** Which unique capability is provided by the Yeast Two-Hybrid system in proteomics?
(A) Quantifying absolute protein molarity
(B) Determining the exact three-dimensional fold of a protein
(C) Identifying in vivo physical protein interactions.
(D) Measuring the isoelectric point of a single peptide

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2008

**225.** A researcher seeking to map the completely unknown metabolic pathway of a newly discovered toxic alkaloid in a plant species would most appropriately utilize:
(A) Simple Single Marker Analysis
(B) Untargeted metabolomics via high-resolution LC-MS
(C) A specific TaqMan SNP assay
(D) Strictly 2D-PAGE

2012

**226.** What physical property does Inductively Coupled Plasma (ICP) exploit to vaporize and ionize sample molecules during ionomic analysis?
(A) Extreme cold (-196°C)
(B) Intense magnetic fields acting on solid metals
(C) Focused visible light lasers
(D) An extremely high-temperature argon plasma torch.

2005

**227.** Which technique is used to identify the binding sites of DNA-binding proteins across the entire genome?
(A) ChIP-seq

into the evolutionary history and structural changes within specific genomic loci.

**44.** (B) To combine multiple resistance genes into a single genotype.
Gene pyramiding is a sophisticated breeding strategy aimed at enhancing the durability and broad-spectrum effectiveness of crop resistance. By using marker-assisted selection, breeders can efficiently combine multiple different resistance genes into a single elite cultivar. This approach makes it much more difficult for pathogens to overcome the host's defenses, as they would need to develop simultaneous mutations to bypass multiple resistance mechanisms.

**45.** (A) Both high-density markers and precise phenotypic data
In genomic selection, the training population serves as the foundation for building accurate prediction models. It must be comprehensively characterized, requiring both high-density molecular marker data and reliable phenotypic measurements for the traits of interest. This dual dataset allows the statistical model to estimate the effect of every marker across the genome, which is then used to predict performance in other populations.

**46.** (C) The marker density required to identify significant associations
The rate at which linkage disequilibrium decays over physical distance is a fundamental factor in the design of Genome-Wide Association Studies. In species where LD decays rapidly, a much higher density of molecular markers is required to ensure that at least one marker is linked to every potential causative locus. Conversely, in species with slow LD decay, fewer markers are needed.

**47.** (D) They demand large DNA quantities and time-consuming procedures.
Traditional hybridization-based markers like RFLPs have significant drawbacks that limit their application in modern high-throughput genotyping. The procedure requires relatively large amounts of high-quality DNA and involves several labor-intensive steps, including restriction digestion, Southern blotting, and probe hybridization. These requirements make it difficult to scale the process for the rapid analysis of thousands of individuals common in contemporary breeding programs.

**48.** (C) To denature double-stranded DNA into single strands
In the Southern blotting protocol, the use of an alkaline transfer buffer serves a critical function beyond providing the medium for movement. The alkaline conditions, typically created by sodium hydroxide, cause the double-stranded DNA fragments within the gel to denature into single strands. This denaturation is essential because the subsequent hybridization step requires single-stranded DNA to pair with the labeled probe.

**49.** (B) Standardizing strict PCR cycling and template concentrations
The sensitivity of RAPD markers to experimental conditions often leads to the appearance of artifactual bands, which can compromise data quality. To improve the reproducibility of the assay, it is essential to implement strict standardization of all PCR parameters. This includes carefully controlling the concentrations of the template DNA and reagents, as well as ensuring consistent thermal cycler performance across different runs.

**50.** (A) I, II, III and V
The Amplified Fragment Length Polymorphism technique is a powerful tool for genome-wide scanning without requiring prior sequence information.
The process involves restriction digestion, adapter ligation, and two rounds of selective amplification. It typically produces dominant marker data, meaning it can detect the presence or absence of fragments but usually cannot distinguish between heterozygotes and homozygotes during standard genetic analysis.

**51.** (A) Frequent dissociation and misalignment at tandem repeats
The high degree of polymorphism observed in Simple Sequence Repeats is largely attributed to the biochemical properties of DNA polymerase during replication. When the polymerase encounters repetitive sequences, it is prone to frequent dissociation and subsequent misalignment with the template strand. This slippage mechanism leads to the expansion or contraction of the repeat array, creating the length variations used as markers.

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**52.** (C) 5' to 3' exonuclease activity of Taq DNA polymerase
TaqMan assays provide a highly specific method for SNP genotyping by utilizing the unique 5' to 3' exonuclease activity of Taq DNA polymerase. During the PCR reaction, if the dual-labeled probe is perfectly hybridized to the target sequence, the advancing polymerase will cleave the probe. This cleavage separates the fluorescent dye from the quencher, resulting in a detectable signal that indicates allele presence.

**53.** (A) Isolating the RAPD fragment, cloning, and sequencing it.
Sequence Characterized Amplified Region markers are developed to convert an anonymous RAPD polymorphism into a robust, locus-specific marker. The protocol begins by excising the target polymorphic band from a RAPD gel. This fragment is then cloned and sequenced to determine its precise primary structure. Based on this sequence, longer and more specific primers are designed to amplify only that particular genomic locus.

**54.** (D) Doubled Haploids (DH)
Doubled Haploid technology provides a rapid method for generating mapping populations where every individual is completely homozygous at all genetic loci. This is achieved by regenerating plants from haploid cells, such as pollen or ovules, followed by spontaneous or induced chromosome doubling. Because they are genetically fixed, DH lines can be evaluated repeatedly across different environments without any risk of further segregation.

**55.** (B) 17.8 cM
The Haldane mapping function is used to estimate genetic distances by accounting for the occurrence of multiple crossovers between markers. It uses the formula $d = -0.5 \ln(1 - 2\theta)$, where $\theta$ is the recombination fraction. For a recombination frequency of 0.15, this mathematical conversion results in a map distance of approximately 17.8 centimorgans, providing a more accurate biological representation.

**56.** (C) Fluorescence in situ hybridization (FISH)
Cytogenetic mapping is a technique used to physically localize genes or DNA markers directly onto the chromosomes. It primarily utilizes fluorescence in situ hybridization, where fluorescently labeled DNA probes are hybridized to metaphase chromosome spreads. By observing the specific locations where the probes bind under a microscope, researchers can determine the physical position of a sequence relative to chromosomal landmarks.

**57.** (D) Two groups representing extremes of the phenotypic distribution
Bulked Segregant Analysis is designed to rapidly identify markers linked to a trait by comparing pooled DNA samples. In an F2 population segregating for disease resistance, the pools are constructed by selecting individuals that exhibit the most extreme phenotypes. One bulk consists of highly resistant plants, while the other contains highly susceptible ones. Markers showing differences between these bulks are likely linked.

**58.** (A) Maximum Likelihood estimation
Interval Mapping is a powerful statistical framework used to identify Quantitative Trait Loci by evaluating the entire space between adjacent markers. It predominantly employs maximum likelihood estimation to calculate the probability that a QTL exists at any given position along the chromosome. This approach provides a more comprehensive and accurate analysis of the genetic architecture of complex traits than single-marker methods.

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**59.** (B) High-throughput sequencing of random fragments and algorithmic assembly.
The Whole Genome Shotgun sequencing strategy is a high-speed approach to genomic characterization that does not require an initial physical map. Genomic DNA is randomly sheared into millions of small fragments, which are then sequenced simultaneously. Advanced bioinformatics algorithms are then used to identify overlaps among the sequences and assemble them into larger, continuous contigs and scaffolds to reconstruct the genome.

**60.** (A) Both A and R are true and R is the correct explanation of A.
Identifying the functional regions of a genome, known as Open Reading Frames, is significantly less complex in prokaryotes than in eukaryotes. Prokaryotic genes are typically continuous sequences that directly code for proteins. In contrast, eukaryotic genes are often interrupted by non-coding introns, requiring sophisticated algorithms to correctly identify splice sites

and exons to predict the final protein product accurately.

**61.** (A) Random insertional mutant libraries
Agrobacterium tumefaciens is widely utilized in functional genomics as a tool for creating insertional mutations. When the bacterium infects plant cells, it transfers a specific segment of its DNA, known as T-DNA, which integrates randomly into the host genome. By generating large populations of these T-DNA insertion lines, researchers can create mutant libraries to systematically study the function of various genes.

**62.** (C) Transcripts Per Million
Transcripts Per Million is a standardized metric used in RNA-Seq analysis to report and compare the expression levels of genes across different samples. It accounts for both the total number of reads generated in the experiment and the length of each specific transcript. By normalizing the data in this way, TPM provides a clear and proportional representation of mRNA abundance in the transcriptome.

**63.** (D) Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS)
Liquid Chromatography coupled with Tandem Mass Spectrometry is considered the gold standard for protein identification in modern proteomics. After proteins are separated and digested into peptides, the LC-MS/MS system provides high-resolution mass measurements and fragmentation patterns. This detailed information allows for the precise determination of the amino acid sequence, enabling the unambiguous identification of proteins even in highly complex biological mixtures.

**64.** (C) The target metabolites are polar, non-volatile, and thermally labile.
Liquid Chromatography-Mass Spectrometry is often the preferred analytical platform for metabolomics when studying compounds that are not suitable for gas chromatography. Many biological metabolites are highly polar, have low volatility, or are sensitive to high temperatures. LC-MS allows these molecules to be separated in a liquid mobile phase at room temperature, ensuring their integrity and allowing for comprehensive profiling.

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**65.** (B) Ionomics
Ionomics is the high-throughput study of the elemental composition of an organism, encompassing all mineral nutrients and trace elements. It is an essential tool for understanding how plants maintain mineral homeostasis under various environmental conditions, such as salt stress. By profiling the ionome, researchers can identify the genetic and physiological mechanisms that regulate the uptake, transport, and accumulation of essential elements.

**66.** (D) Collinearity
Collinearity is a fundamental concept in comparative genomics that describes the conservation of the linear order of genes along chromosomes across different species. This structural relationship is often observed between closely related taxa, such as different grass species. Identifying collinear regions allows researchers to transfer genetic information from well-studied model organisms to more complex crops, facilitating gene mapping and evolutionary studies.

**67.** (A) Cross-over events flanking the target locus to minimize linkage drag.
Recombinant selection is a critical step in marker-assisted breeding aimed at reducing the introgressed segment size around a target gene. The primary goal is to identify individuals that have experienced crossover events very close to the locus of interest. This minimizes linkage drag, which is the co-inheritance of undesirable donor parent alleles that can negatively affect the performance of the line.

**68.** (C) Summing predicted effects of all genome-wide markers simultaneously.
Genomic Estimated Breeding Values are calculated in genomic selection by integrating information from thousands of molecular markers distributed across the entire genome. Statistical models estimate the individual effect of each marker on the target trait using a training population. To determine the GEBV of a new individual, these predicted effects are summed, providing a comprehensive estimate of its genetic merit.

**69.** (A) False-positive associations due to relatedness
In Genome-Wide Association Studies, the presence of genetic relatedness among individuals can lead to serious statistical errors. If the

**105.** (C) Inability to resolve complex repetitive regions due to short read lengths.
Short-read sequencing technologies, like Illumina, face a major challenge when assembling genomes that contain many repetitive elements. Because the reads are much shorter than many common repetitive sequences, it is difficult for bioinformatics algorithms to determine their correct position and orientation. This often leads to fragmented assemblies where repetitive regions are collapsed or represented as gaps between continuous contigs.

**106.** (A) Start codons, stop codons, and splice site donor/acceptor sequences
Accurate structural genome annotation in eukaryotes requires the identification of specific molecular signals that define gene boundaries. Algorithms must recognize start and stop codons to define the open reading frame. Furthermore, because eukaryotic genes contain introns, the detection of highly conserved donor and acceptor splice site sequences is essential for correctly predicting the exon-intron structure of the transcript.

**107.** (B) G/C to A/T transitions
Ethyl methanesulfonate is a widely used chemical mutagen in forward genetics due to its efficiency and predictability. EMS functions by alkylating guanine bases, which causes them to mispair with thymine during DNA replication. This chemical process predominantly results in G/C to A/T transition mutations. These single-base changes often create functional variations that researchers can screen for to study gene function.

**108.** (A) Complementary base-pairing between cDNA and immobilized probes
Microarray technology is a transcriptomic tool that relies on the fundamental physical principle of nucleic acid hybridization. The chip contains thousands of immobilized, single-stranded DNA probes with known sequences. When fluorescently labeled cDNA from a sample is applied, it will bind to its exact complementary probe through base-pairing. The intensity of the resulting signal reflects the abundance of the transcript.

**109.** (A) Mass-to-charge ($\frac{m}{z}$) ratio
In Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry, the time it takes for an ionized peptide to travel through a vacuum tube is measured. This time-of-flight is directly proportional to the square root of the peptide's mass-to-charge ratio. By precisely timing this flight, the instrument can determine the accurate mass of the peptide, which is used for protein identification.

**110.** (A) Both A and R are true and R is the correct explanation of A.
Chemical derivatization is a critical preparatory step in the GC-MS analysis of many biological metabolites.
Many metabolites are highly polar and non-volatile, making them unsuitable for gas chromatography. Derivatization reagents replace active hydrogens with more stable chemical groups, which increases the volatility and thermal stability of the molecules. This process ensures that the compounds can be effectively separated in the gas phase.

**111.** (D) Helium (He)
Ionomics focuses on the comprehensive profiling of essential nutrients and trace elements within an organism's tissues. Standard profiles typically include elements such as zinc, iron, and manganese, which are critical for various biological processes. Helium, being an inert noble gas that does not participate in biological systems or accumulate in plant tissues, would not be part of a standard ionomic profile.

**112.** (C) Macrosynteny
Macrosynteny refers to the conservation of gene content and relative order across large chromosomal segments between different species. This large-scale structural relationship is a common feature in the genomes of related taxa, like Arabidopsis and Brassica. While internal rearrangements and duplications occur over evolutionary time, the overall organization of these conserved blocks provides vital information for comparative genomic studies.

**113.** (B) Developing durable resistance by accumulating multiple R-genes
Gene pyramiding is a strategic application of marker-assisted selection intended to create more robust and long-lasting disease resistance. By combining multiple resistance genes with different mechanisms into a single cultivar, breeders create a multi-layered defense system. This makes it significantly more difficult for a pathogen to evolve and overcome the host's

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recurrent parent's genome increases. By the BC6 generation, individuals have typically recovered more than 99% of the recurrent parent's genetic background, with the exception of the small chromosomal segment containing the target gene from the donor parent.

**79.** (D) Meiotic crossing-over
Linkage mapping is a fundamental genetic tool used to determine the relative positions of genes and markers on a chromosome. It relies on analyzing the frequency of meiotic crossing-over events that occur between loci. The closer two loci are physically located, the less likely they are to be separated by a crossover, resulting in a lower observed recombination frequency during genetic analysis.

**80.** (C) I and III
Genetic and physical maps provide different but complementary representations of the genome.
Genetic map distances are measured in centimorgans, while physical maps use base pairs. The relationship between these two measures is not constant across a chromosome, as recombination rates vary significantly between regions like centromeres and telomeres. Therefore, stating that genetic distance is measured in base pairs is incorrect.

**81.** (B) False-positives due to random sampling errors
In Bulked Segregant Analysis, the number of individuals pooled into each bulk is a critical parameter. If the bulk size is too small, the analysis becomes highly susceptible to random sampling errors. This can lead to the identification of false-positive markers that appear linked to the trait purely by chance, rather than due to actual physical proximity on the chromosome to the target gene.

**82.** (D) Multiple Interval Mapping (MIM)
Multiple Interval Mapping is an advanced statistical approach for QTL analysis that offers greater precision than simpler methods. It explicitly models the presence of several QTLs simultaneously across the genome. By accounting for the effects of multiple loci at once, MIM reduces the residual variance in the model, improves the power to detect small-effect QTLs, and allows for the evaluation of epistasis.

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**83.** (A) Massive parallelization of sequencing reactions to increase throughput.
The shift from Sanger sequencing to Next-Generation Sequencing has revolutionized genomics by vastly increasing data output. NGS platforms achieve this by performing millions of sequencing reactions simultaneously in a highly parallelized manner. This massive scale allows researchers to sequence entire genomes in a fraction of the time and at a significantly lower cost per base than was possible with traditional methods.

**84.** (C) A codon sequence bounded by a start and stop codon.
An Open Reading Frame is a continuous stretch of DNA that has the potential to be translated into a protein. In bioinformatics, an ORF is identified as a sequence of nucleotides that begins with a start codon and ends with a stop codon, without any intervening stop codons. Identifying ORFs is a fundamental step in the structural annotation of genomes for gene discovery.

**85.** (A) Generating gene knockouts using the CRISPR-Cas9 system
Reverse genetics is a strategic approach used to determine the biological function of a specific gene by altering its expression. The CRISPR-Cas9 system is a classic reverse genetics tool that allows researchers to create precise, targeted gene knockouts. By disrupting the gene sequence and observing the resulting phenotypic changes, scientists can gain direct insights into the role that the gene plays.

**86.** (D) Comprehensive characterization and quantification of transcriptomes
RNA-Seq is a powerful transcriptomic application that utilizes high-throughput sequencing to analyze the full complement of RNA in a biological sample. It provides a comprehensive view of gene expression, allowing for the discovery of novel transcripts, the identification of alternative splicing events, and the precise quantification of mRNA abundance. This technology is essential for understanding how gene activity changes under different conditions.

**87.** (B) The DNA-binding domain (DBD) and activation domain (AD)
The Yeast Two-Hybrid system is a widely used proteomics tool for detecting physical interactions between proteins. It relies on the

reconstitution of a functional transcription factor from two separate parts: a DNA-binding domain and an activation domain. When the two target proteins interact, these domains are brought into close proximity, allowing them to initiate the transcription of a reporter gene in the host.

**88.** (C) By employing a suite of orthogonal analytical platforms
The vast chemical diversity of the metabolome presents a significant challenge for comprehensive profiling. No single analytical technique can detect every metabolite in a sample. To address this, researchers often employ a suite of orthogonal platforms, such as GC-MS and LC-MS. By combining different separation and detection methods, they can maximize the range of metabolites identified and provide a more complete profile.

**89.** (D) Regulation of mineral nutrient uptake mechanisms
Ionomics is the comprehensive study of the elemental composition of an organism. It provides direct insights into the physiological processes that regulate the uptake, transport, and accumulation of mineral nutrients. By profiling the ionome under different environmental or genetic conditions, researchers can identify the specific transporters and regulatory networks that maintain mineral homeostasis and help plants adapt to stresses like nutrient deficiency or toxicity.

**90.** (D) I, II and IV
Comparative genomics explores the evolutionary relationships between genes and genomes.
**Evolutionary Gene Categories:**
I. Paralogues arise through duplication within a single lineage.
II. Orthologues result from speciation and often share functions.
III. Macrosynteny describes large-scale gene order, not single-nucleotide level.
IV. Xenologues are acquired through horizontal gene transfer between different species.

**91.** (B) Using markers tightly linked to target genes to identify carriers.
Foreground selection is a fundamental component of marker-assisted selection aimed at ensuring the presence of a specific target gene. It involves using molecular markers that are very tightly linked to the gene of interest to identify which individuals in a population carry the desired allele. This allows breeders to select the correct genotypes at an early stage, regardless of environmental influence on phenotype.

**92.** (A) High genetic relatedness and linkage disequilibrium with the breeding population.
Training population optimization is critical for maximizing the accuracy of genomic selection models. To be effective, the training population must share high genetic relatedness and similar patterns of linkage disequilibrium with the breeding population. This ensures that the marker effects estimated in the training set are biologically relevant and will accurately predict the performance of individuals in the selection population.

**93.** (A) Higher than that of standard biparental linkage mapping.
Genome-Wide Association Studies offer significantly higher mapping resolution than traditional biparental mapping. This is because GWAS exploits historical recombination events that have accumulated over many generations in a diverse natural population. These numerous crossovers break the genome into much smaller segments, allowing researchers to pinpoint the location of genes or QTLs with much greater precision than is possible in recent biparental crosses.

**94.** (C) Dominant
A molecular marker that exhibits a 3:1 segregation ratio for presence versus absence in an F2 population follows a classic Mendelian inheritance pattern for a single dominant locus. This ratio indicates that the marker is dominant, as individuals that are either homozygous dominant or heterozygous for the band show the same phenotype, while only homozygous recessive individuals lack the specific band.

**95.** (D) Substitution within the recognition palindrome
RFLP depends on restriction enzymes recognizing specific palindromic DNA sequences. A substitution within the recognition palindrome alters the enzyme's binding site, preventing cleavage at that location. This changes the lengths of restriction fragments, producing a different banding pattern. Mutations outside the recognition sequence, genome duplication, or

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alternative splicing do not directly eliminate a restriction enzyme recognition site.

**96.** (C) Binding to closely spaced, inverted complementary sequences.
RAPD uses short, arbitrary primers that anneal at multiple sites throughout the genome. Amplification occurs only when two primer-binding sites are closely spaced and oriented toward each other on opposite DNA strands. This generates discrete PCR fragments without prior sequence knowledge. RAPD does not target poly-A tails, repetitive DNA exclusively, or depend on restriction enzyme cleavage.

**97.** (B) One
In AFLP, the pre-amplification PCR employs primers complementary to the adapter and restriction site sequences, typically containing one selective nucleotide at the 3′ end. This moderately reduces the number of amplified fragments while maintaining broad genome coverage. A later selective amplification step uses additional selective nucleotides to further improve resolution and reproducibility.

**98.** (D) Short repetitive dinucleotides misalign more easily during replication.
Dinucleotide repeats are highly susceptible to replication slippage because their short, repetitive units readily misalign during DNA synthesis. This causes insertions or deletions of repeat units, leading to microsatellite length variation. Longer repeat motifs, such as hexanucleotides, are generally more stable and less likely to undergo such misalignment during replication.

**99.** (A) They are afflicted by ascertainment bias by only genotyping known SNPs.
A major limitation of SNP arrays is ascertainment bias, as they detect only previously identified SNPs included on the chip. Novel or rare variants are not discovered, limiting genetic diversity studies. However, SNP arrays can genotype hundreds of thousands of markers simultaneously and accurately distinguish homozygous from heterozygous genotypes without using radioisotopes.

**100.** (A) II and V
Mapping populations vary in their structure and application. RILs are produced through many generations of selfing, thus capturing numerous meiotic recombinations, unlike the single generation in F2s. Near Isogenic Lines differ primarily at a small targeted chromosomal segment, not at the entire mitochondrial level. Therefore, statements II and V are fundamentally incorrect descriptions of these genetic resources in plant breeding.

**101.** (A) Independent assortment of unlinked genes yields 50% recombinant gametes.
Recombination frequency is mathematically limited to a maximum of 0.5 because of the principle of independent assortment. For genes located on different chromosomes or very far apart on the same chromosome, the probability of any specific gamete being recombinant is 50%. Even with multiple crossovers occurring between two very distant markers, the average frequency of recombinant types will never exceed this theoretical threshold.

**102.** (D) It drastically decreases because recombination is heavily suppressed.
In centromeric heterochromatin, the ratio of genetic distance to physical length is very low compared to other chromosomal regions. This occurs because the highly condensed nature of the DNA in the centromere significantly suppresses the occurrence of meiotic crossing-over. Consequently, a large physical segment of DNA in these regions will correspond to a very small distance on a genetic linkage map.

**103.** (B) The SNP-index (allele frequency difference) between contrasting pools
QTL-seq is a powerful method that combines Bulked Segregant Analysis with Next-Generation Sequencing to rapidly map QTLs. The primary metric used is the SNP-index, which represents the frequency of parental alleles in each bulked pool. By calculating the difference in SNP-index between the contrasting bulks across the genome, researchers can identify the specific genomic regions where the pools significantly diverge.

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**104.** (C) It cannot distinguish tightly linked small-effect QTLs from loose large-effect QTLs.
Single Marker Analysis has a significant statistical limitation because it only evaluates one marker at a time. It cannot independently estimate the physical location of a QTL and its effect size. This confounding means that a large-effect QTL located far from the marker can produce the same statistical result as a small-effect QTL that is very tightly linked.

orientation at both ends of the target region. Because these repeats are found throughout the genome, the resulting PCR products provide a multi-locus profile that is highly polymorphic.

**123.** (B) Successive generations of strict self-pollination
A Recombinant Inbred Line population is developed through a systematic process of inbreeding. Starting from an F2 population, individuals undergo several successive generations of strict self-pollination. This process progressively increases homozygosity while capturing numerous recombination events that occurred during the multiple rounds of meiosis. By the sixth or seventh generation, the lines are genetically fixed and represent a permanent mapping resource.

**124.** (B) Crossover interference
The Kosambi mapping function is an improvement over simpler models for estimating genetic map distances. Unlike the Haldane function, which assumes that crossovers occur independently, the Kosambi function accounts for crossover interference. This biological phenomenon describes the tendency of one crossover to inhibit the formation of another nearby crossover, a reality that must be modeled for accurate genetic mapping.

**125.** (A) Marker anchors mapped onto sequenced assemblies
Integrating a genetic linkage map with a physical sequence map requires the identification of specific anchor points. These anchors are typically molecular markers that have both a known position on the genetic map, determined by recombination frequencies, and a known location within the physical DNA sequence. By aligning these common points, researchers can correlate genetic distances with actual physical lengths.

**126.** (C) It identifies loci within wide chromosomal intervals.
While Bulked Segregant Analysis is an efficient method for identifying markers linked to a trait, it has a significant limitation in terms of resolution. Because it relies on the comparison of only two pooled samples, it typically identifies genetic regions within relatively wide chromosomal intervals. Further fine-mapping using a larger number of individuals and markers is usually required to pinpoint the exact gene.

**127.** (D) Composite Interval Mapping (CIM)
Composite Interval Mapping is a sophisticated statistical strategy that enhances the precision of QTL detection. It achieves this by combining interval mapping with multiple regression analysis. By incorporating markers outside the target interval as cofactors, the model can account for and remove the variance contributed by other QTLs. This reduces the background noise and increases the accuracy of locating the target QTL.

**128.** (C) Average number of times a nucleotide is sequenced.
Sequencing depth, or coverage, is a fundamental metric in Next-Generation Sequencing that describes the thoroughness of the analysis. It represents the average number of times that each specific nucleotide in the target genome is represented in the final set of sequence reads. Higher depth increases the accuracy of base calling and allows for the reliable detection of rare genetic variants.

**129.** (B) Variable-length introns and alternative splicing.
Predicting the structure of eukaryotic genes is mathematically challenging due to the complex nature of their genomic organization. Genes are frequently interrupted by introns of varying lengths, and the process of alternative splicing can result in multiple different mRNA products from a single locus. Bioinformatics algorithms must account for these variables to accurately identify exon boundaries and predict the final protein sequence.

**130.** (A) I and II
Functional genomics uses various tools to investigate the relationship between genes and phenotypes.
**Evaluation of Functional Tools:**
I. Transposon tagging utilizes DNA insertions to disrupt gene function.
II. RNAi employs double-stranded RNA to target and degrade specific mRNA.
III. T-DNA insertion is typically random and not targeted to specific loci.

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resistance, as it would need to bypass all accumulated defenses simultaneously.

**114.** (D) All genome-wide markers used to calculate breeding values
Genomic selection is a modern breeding approach that differs from traditional marker-assisted selection by its comprehensive use of genetic data. Instead of focusing on just a few significant markers linked to major QTLs, genomic selection utilizes all available markers across the entire genome. These markers are used simultaneously to calculate breeding values, allowing for the selection of complex traits controlled by many small-effect loci.

**115.** (A) Meiotic recombination
Linkage disequilibrium refers to the non-random association of alleles at different loci within a population. Over successive generations, the primary mechanism that causes LD to decay is meiotic recombination. During meiosis, crossovers occur between homologous chromosomes, which breaks up existing allelic combinations. The frequency of these events over time leads to a more random distribution of alleles, reducing the observed levels of LD.

**116.** (C) They exhibit strictly maternal inheritance.
In most angiosperms, the chloroplast genome is inherited exclusively through the maternal lineage, as the organelles are typically excluded from the pollen during fertilization. This uniparental inheritance pattern makes chloroplast DNA markers unsuitable for tracking gene flow that occurs through pollen dispersal. While useful for studying maternal lineages, they do not provide information on the contributions of the paternal parent to the next generation.

**117.** (A) The large fragment is cleaved into two smaller ones, altering banding.
RFLP markers detect variations in DNA sequences that affect the recognition sites of restriction enzymes. If a point mutation creates a new restriction site within what was previously a single, large DNA fragment, the enzyme will now cut the DNA at that location. This results in the original large band disappearing and being replaced by two smaller fragments, altering the observed banding pattern.

**118.** (D) RAPD
Randomly Amplified Polymorphic DNA is a marker system that requires no prior sequence information for the target genome. It utilizes short, arbitrary primers to amplify random regions of the DNA. This makes it a very accessible and cost-effective tool for studying poorly characterized species. In contrast, markers like SSRs and CAPS require specific sequence data to design primers for target-specific amplification.

**119.** (B) T4 DNA Ligase
The AFLP technique involves several specific biochemical steps to generate DNA polymorphisms. After the genomic DNA is digested by restriction endonucleases to create sticky ends, synthetic double-stranded adapters must be attached to these ends. This critical ligation step is performed by T4 DNA ligase, which facilitates the formation of phosphodiester bonds between the restriction fragments and the known adapter sequences.

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**120.** (A) P-3, Q-1, R-2, S-4
Simple Sequence Repeats are versatile molecular markers used extensively in genetic research.
**SSR Concepts and Descriptions:**
P. Slippage is the biochemical mechanism for repeat variation.
Q. EST-SSRs are derived from transcribed mRNA sequences.
R. Flanking regions are conserved for primer design.
S. Genomic SSRs originate from non-coding chromosomal DNA.

**121.** (D) Two
In a diploid organism, each individual possesses two copies of every chromosome, one inherited from each parent. For any specific Single Nucleotide Polymorphism locus, an individual can have at most two different alleles. These can be the same, representing a homozygous state, or different, representing a heterozygous state. This biallelic nature is a fundamental characteristic of SNPs in diploid genetics.

**122.** (A) Inter-Simple Sequence Repeats (ISSR)
Inter-Simple Sequence Repeat markers are generated by amplifying the DNA segments that lie between two adjacent microsatellite repeats. This technique utilizes a single primer based on a repeat motif, which binds in an inverted

kinship matrix is not included in the mixed linear model, the analysis may produce numerous false-positive associations. These spurious results occur because the model incorrectly attributes phenotypic similarities to specific markers rather than to the overall shared genetic background of the related individuals.

**70.** (A) P-3, Q-2, R-4, S-1
Molecular markers are characterized by different underlying biochemical principles and provide varying levels of genetic information.
**Marker Characteristics:**
P. RFLP uses restriction digestion and hybridization.
Q. RAPD employs short arbitrary primers.
R. SSR targets tandem repeat variations.
S. SNP detects single-base changes and is highly abundant.

**71.** (B) They require significantly lesser amounts of template DNA.
One of the most significant advantages of PCR-based markers over traditional hybridization methods is their exceptional sensitivity. Because the target DNA sequence is geometrically amplified during the PCR process, only a very small amount of starting template is required. This efficiency allows researchers to perform genetic analyses using minimal tissue samples, which is particularly beneficial for large-scale screenings and high-throughput genotyping.

**72.** (C) Radioisotope-labeled probe (e.g., $^{32}P$)
Visualizing specific DNA sequences in an RFLP analysis traditionally requires the use of radioisotope-labeled probes. These probes are designed to be complementary to the target sequence and are typically labeled with phosphorus-32. When the probe hybridizes to the membrane-bound DNA, it emits radiation that exposes an X-ray film, producing the characteristic banding patterns seen on an autoradiograph for genetic interpretation.

**73.** (D) The length of the genomic DNA extraction protocol
The reproducibility of RAPD assays is highly sensitive to the precise conditions within the PCR reaction. Factors such as thermal cycler ramp rates, magnesium chloride concentrations, and the specific brand of DNA polymerase can all alter the resulting banding patterns. However, the duration of the DNA extraction protocol itself is unlikely to impact results, provided the final DNA is pure.

**74.** (A) MseI is a 4-base cutter generating small fragments compared to EcoRI.
In the AFLP procedure, the choice of restriction enzymes is critical for generating a suitable fragment distribution. MseI is a frequent cutter because it recognizes a four-base sequence, resulting in many small DNA fragments. EcoRI is a rare cutter that recognizes a six-base sequence, producing fewer, larger fragments. This combination ensures that most fragments carry an MseI end, facilitating efficient amplification.

**75.** (B) Conserved flanking regions uniquely surrounding the SSR locus.
To successfully amplify a Simple Sequence Repeat locus, primers must be designed to bind to the unique, non-repetitive DNA sequences that surround the repeat array. These flanking regions are typically highly conserved within a species, allowing the same set of primers to be used across different individuals. The variation in the number of repeat units between these primers results in the length polymorphisms.

**76.** (B) Allele-specific oligonucleotide hybridization
SNP microarrays are high-throughput tools that allow for the simultaneous genotyping of thousands of loci. They function based on the principle of allele-specific oligonucleotide hybridization. The chip contains immobilized probes that perfectly match different SNP alleles. When labeled genomic DNA is hybridized to the chip, it binds only to its exact match, and the resulting signal is captured to determine the genotype.

**77.** (A) The 3' or 5' end of a microsatellite repeat sequence.
The Inter-Simple Sequence Repeat technique is a powerful method for generating DNA polymorphisms without requiring prior genomic sequence information. It utilizes a single primer that is complementary to a microsatellite repeat motif. By adding a short "anchor" of one or two nucleotides at the 3' or 5' end, the primer is forced to bind at the exact edge of the repeat.

**78.** (D) BC6
Near Isogenic Lines are developed through a process of repeated backcrossing to a recurrent parent. In each generation, the percentage of the

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**131.** (A) To sequence multiple short tags simultaneously.
Concatemerization is a central step in the Serial Analysis of Gene Expression protocol. After capturing short sequence tags from mRNA transcripts, these tags are ligated together to form long chains, or concatemers. This approach allows multiple unique tags to be sequenced in a single reaction, significantly increasing the throughput and efficiency of measuring gene expression levels within a sample.

**132.** (C) Its net electrical charge is zero, stopping migration.
In the first dimension of 2D-PAGE, proteins are separated by isoelectric focusing within a pH gradient. Each protein will migrate through the gel until it reaches its specific isoelectric point. At this precise pH, the protein's net electrical charge becomes zero. Because it no longer experiences an electrostatic force from the electrical field, its movement stops, resulting in high-resolution separation.

**133.** (A) Ion deflection by fields proportional to $\frac{m}{z}$ ratio
The separation of molecules in a mass spectrometer occurs after they have been ionized and accelerated into a mass analyzer. The analyzer uses electrical or magnetic fields to deflect the ions as they move. The degree of deflection is directly proportional to the ion's mass-to-charge ratio. This physical principle allows the instrument to sort and detect different molecules based on their unique $\frac{m}{z}$ values.

**134.** (D) High-throughput simultaneous detection of trace isotopes.
Inductively Coupled Plasma Mass Spectrometry is the preferred platform for ionomics due to its superior analytical capabilities. Unlike traditional flame-based methods, ICP-MS can simultaneously detect and quantify almost all elements in the periodic table at very low concentrations. This high-throughput capacity and extreme sensitivity for trace isotopes make it ideal for the comprehensive elemental profiling required in mineral homeostasis studies.

**135.** (B) Whole genome or segmental duplication events
Paralogous genes are members of a gene family that have evolved through duplication events within the same lineage. These duplications can involve single genes, large chromosomal segments, or even the entire genome. Over time, the duplicated copies may evolve new functions or patterns of expression, contributing to the genetic diversity and evolutionary complexity of the organism.

**136.** (C) Linkage drag from undesirable flanking alleles
Recombinant selection is a critical step in marker-assisted backcrossing designed to refine the introgressed genomic segment. The goal is to identify individuals with crossover events very close to the target gene to eliminate undesirable donor parent alleles. This process effectively minimizes linkage drag, which can otherwise bring in negative traits that reduce the overall quality and performance of the elite line.

**137.** (C) Prediction accuracy
In genomic selection, the performance of a model is evaluated by its prediction accuracy. This parameter measures the correlation between the calculated Genomic Estimated Breeding Values and the true breeding values of the individuals. High prediction accuracy is essential for making effective selection decisions in breeding programs, allowing for the rapid improvement of complex traits without extensive phenotypic testing.

**138.** (A) Q-matrix (population structure matrix)
Population stratification is a significant confounding factor in Genome-Wide Association Studies that can lead to false-positive results. To correct for this, researchers include a Q-matrix in their statistical models to account for the underlying subgroup structure of the population. This adjustment ensures that the identified associations between markers and traits are truly due to genetic linkage rather than shared ancestry.

**139.** (B) Diversity Arrays Technology (DArT)
Diversity Arrays Technology is a high-throughput molecular marker system that relies on DNA hybridization rather than specific PCR amplification. It uses microarrays containing thousands of genomic fragments to detect polymorphisms across the entire genome simultaneously. This method is particularly useful for genetic mapping and diversity studies in species with limited sequence information, as it provides a broad genomic overview.

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**175.** (D) BLAST (Basic Local Alignment Search Tool)
BLAST is one of the most fundamental and widely used bioinformatics tools in genomics. It allows researchers to compare a novel DNA or protein sequence against vast databases of known sequences to identify regions of similarity. This capability is essential for genome annotation, as sequence homology provides vital clues about the potential function and evolutionary origin of newly discovered genes and genomic features.

**176.** (C) Disrupting loci with known sequences to tag mutants.
Insertional mutagenesis is a powerful forward genetics strategy used to identify genes associated with specific traits. It involves the random integration of known DNA sequences, such as T-DNA or transposons, into the genome. When an insertion disrupts a functional gene, it creates a mutant phenotype. Because the sequence of the insert is known, it serves as a molecular tag for rapidly identifying the causative gene.

**177.** (B) Mapping quality-filtered reads against a reference.
The bioinformatics analysis of RNA-Seq data begins with a critical step to organize the massive amount of sequence information. After filtering the raw reads for quality, they are mapped against a reference genome or transcriptome. This alignment process allows researchers to determine the genomic origin of each read. The number of reads that map to each gene is then used to quantify transcript expression.

**178.** (D) Resolution of hydrophobic and extreme-weight proteins.
While 2D-PAGE is a powerful tool for protein separation, it has significant limitations that LC-MS-based proteomics can overcome. Hydrophobic proteins and those with extremely high or low molecular weights are often poorly resolved or lost during the 2-D gel process. Shotgun proteomics bypasses these issues by digesting the entire protein mixture into peptides, which are then more easily separated and identified by liquid chromatography.

**179.** (A) Replacing active hydrogens to increase volatility.
Chemical derivatization is an essential preparatory step for the GC-MS analysis of many polar biological metabolites. Reagents such as MSTFA work by replacing active hydrogen atoms in functional groups with more stable, non-polar silyl groups. This chemical modification significantly increases the volatility and thermal stability of the molecules, allowing them to be effectively separated and analyzed in the high-temperature gas phase.

**180.** (B) Plant tissue sampling → Acid digestion → Ionization → Detection
Elemental profiling in ionomics requires a systematic series of operational steps to ensure accurate and precise results.
**Ionomic Analysis Workflow:**
The process begins with the careful sampling of plant tissues. These samples are then subjected to acid digestion to break down organic matter and release the minerals into solution. This liquid is then nebulized and introduced into the mass spectrometer for ionization and subsequent detection of the constituent elements.

**181.** (A) Orthologues
Orthologues are homologous genes in different species that have diverged from a common ancestral gene through a speciation event. Because they are directly descended from the same gene in the last common ancestor, orthologues typically retain the same biological function across different organisms. Identifying orthologous relationships is a central task in comparative genomics for understanding the evolutionary conservation of genetic pathways and traits.

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**182.** (D) Multiplexing three co-dominant markers for each gene.
In gene pyramiding, phenotypic selection alone is often insufficient because different resistance genes may produce identical outward appearances. To confirm that an individual carries multiple specific genes, breeders use marker-assisted selection. By multiplexing several co-dominant markers, each linked to a different resistance locus, they can simultaneously verify the presence of every target gene in a single genotype, ensuring the successful accumulation of resistance.

**183.** (B) Sum of additive genetic effects from genome-wide markers.
Genomic Estimated Breeding Values are the central output of genomic selection models. They represent a comprehensive estimate of

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overview of the genome's organization and structure.

**149.** (A) Genetically unlinked to the resistance trait.
In Bulked Segregant Analysis, markers are evaluated for their association with a target trait by comparing pooled DNA samples. A marker that is linked to the trait will show a significant difference in allele frequency between the two bulks. If a marker appears with equal frequency in both the resistant and susceptible bulks, it indicates that the locus is genetically unlinked.

**150.** (A) I, II, III and V
QTL mapping utilizes various statistical methods to identify the genetic regions controlling quantitative traits.
**QTL Mapping Methodologies:**
I. SMA evaluates individual markers without map distance.
II. IM uses flanking markers for interval analysis.
III. CIM incorporates cofactors to control background variance.
IV. IM is still susceptible to the effects of ghost QTLs.
V. Reliable QTL mapping requires both high-quality genotypic and phenotypic data.

**151.** (B) Constructing and mapping BAC libraries was labor-intensive.
Hierarchical Clone-by-Clone sequencing was the standard approach for early genome projects, but it had significant drawbacks. The process required the creation and physical mapping of massive BAC libraries, which was extremely labor-intensive and time-consuming. This logistical bottleneck led to the development and widespread adoption of Whole Genome Shotgun sequencing, which could characterize genomes much more rapidly by sequencing random fragments.

**152.** (C) Assemblies collapsing into fragmented contigs.
Repetitive DNA sequences present a major challenge for bioinformatics algorithms during genome assembly. Because many reads from different parts of the genome appear identical, it is difficult to determine their true position. This ambiguity often causes the assembly to collapse repetitive regions together or fail to connect contigs, resulting in a highly fragmented genome sequence with numerous gaps.

**153.** (D) Specific gene transcript is needed for structural integrity.
RNA interference is a reverse genetics tool that reduces the expression of a target gene by degrading its mRNA. When an RNAi experiment targeting a lignin-biosynthetic gene results in plants with weak stems, it provides direct evidence of the gene's function. The observed phenotype confirms that the presence of the gene's transcript is essential for the structural integrity of the plant.

**154.** (A) Microarray uses predefined probes; RNA-Seq sequences all.
Microarray and RNA-Seq are both used for transcriptomic analysis, but they function on different principles. Microarrays rely on the hybridization of cDNA to a set of predefined probes fixed to a chip, meaning they can only detect known sequences. In contrast, RNA-Seq uses high-throughput sequencing to characterize every RNA molecule in the sample, allowing for the discovery of novel transcripts and variants.

**155.** (C) The nucleus
The Yeast Two-Hybrid system is a molecular assay designed to detect protein-protein interactions within a living cell. For the system to work, the interaction between the target proteins must occur within the nucleus of the yeast host. This localization is necessary because the interaction reconstitutes a functional transcription factor that must bind to a promoter to initiate the expression of a reporter gene.

**156.** (B) Water (samples must be highly desiccated)
Sample preparation is a critical step in metabolomics, particularly when using GC-MS. The extraction protocol requires the complete removal of water from the sample, as moisture can interfere with the chemical derivatization process and damage the analytical column. High levels of desiccation ensure that the metabolites are in a stable form and are suitable for the subsequent high-temperature gas phase analysis.

**157.** (A) Direct accumulation and distribution of minerals.
Ionomic profiling using ICP-MS provides unique insights into the mineral status of an organism that cannot be obtained through transcriptomics alone. While transcriptomics measures gene activity, ionomics directly quantifies the actual accumulation and distribution of essential

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elements and trace minerals within tissues. This direct measurement is vital for understanding the final physiological outcome of the complex regulatory networks that maintain mineral homeostasis.

**158.** (C) Conservation of linear gene order along chromosomes.
Collinearity is a central concept in comparative genomics that describes the preservation of the linear sequence of genes on a chromosome across different species. This conservation often persists over long evolutionary periods, especially in related groups like the grasses. Identifying collinear regions allows researchers to use information from well-sequenced model organisms to assist in the mapping and characterization of genes in other species.

**159.** (D) Precise markers linked to the specific target gene.
Foreground selection is a focused approach in marker-assisted breeding intended to ensure the inheritance of a specific desired gene. The breeder's primary focus is on utilizing molecular markers that are very tightly linked to the target locus. This allow for the efficient identification of individuals that carry the specific gene of interest, regardless of the rest of their genetic background.

**160.** (C) A is true but R is false.
Genomic selection is a powerful tool for improving complex traits that are controlled by many small-effect loci.
Unlike traditional marker-assisted selection, which focuses on identifying and moving a few major QTLs, genomic selection uses all markers across the genome simultaneously to predict performance. Therefore, while genomic selection is highly effective for complex traits, the reason provided regarding major markers is incorrect.

**161.** (C) Spurious marker-trait associations.
Population structure is a major confounding factor in Genome-Wide Association Studies. If a study population contains distinct subgroups with different ancestral backgrounds, these differences can result in spurious associations between markers and traits. Failure to statistically account for this structure can lead to numerous false-positive results, where markers appear linked to a trait purely due to shared ancestry rather than actual genetic linkage.

**162.** (D) They are visually scorable in the field without equipment.
Morphological markers were the first tools used in genetic mapping and still offer a unique advantage in certain breeding scenarios. Unlike molecular markers, which require specialized laboratory equipment and procedures, morphological markers are based on observable physical traits such as flower color or leaf shape. This allows them to be quickly and easily scored directly in the field by visual inspection.

**163.** (A) RFLP
The RFLP technique has several demanding requirements that limit its high-throughput potential. One of the primary bottlenecks is the need for large quantities of highly pure, high-molecular-weight genomic DNA. This is necessary because the DNA must be digested by restriction enzymes and then separated as intact fragments for Southern blotting and hybridization, making the protocol more sensitive to DNA quality.

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**164.** (C) Dominant inheritance cannot distinguish AA from Aa.
RAPD markers are characterized by dominant inheritance, meaning that they only indicate the presence or absence of a specific DNA fragment. In an F2 population, individuals that are homozygous dominant or heterozygous for the marker will produce the same band, while only homozygous recessive individuals will lack it. This inability to distinguish between the two states prevents the accurate calculation of allele frequencies.

**165.** (A) High-resolution denaturing polyacrylamide gels (PAGE)
The AFLP technique produces a large number of DNA fragments of varying lengths in a single reaction. To resolve these complex profiles, high-resolution denaturing polyacrylamide gel electrophoresis is traditionally used. PAGE provides the necessary sensitivity to separate fragments that differ by only a single nucleotide in length, allowing for the clear and precise visualization of the numerous polymorphic bands generated by the assay.

**166.** (D) Taq polymerase slippage across tandem repeats
Stutter bands are a common artifact observed when amplifying SSR loci using PCR. These bands appear as additional, slightly shorter

fragments adjacent to the main product. They are primarily caused by Taq polymerase slippage, where the enzyme dissociates and reattaches incorrectly within the tandem repeat array during the amplification cycles. This slippage leads to the formation of minor products with different repeat counts.

**167.** (B) Transversion
Single Nucleotide Polymorphisms are classified based on the nature of the nucleotide substitution. A transversion is a specific type of mutation where a pyrimidine base, such as Cytosine or Thymine, is replaced by a purine base, like Adenine or Guanine, or vice versa. These mutations are less common than transitions but are important sources of genetic variation that are analyzed in genomics.

**168.** (C) Designing longer specific primers based on the sequence.
Converting an anonymous RAPD marker into a robust SCAR marker is a critical step for improving specificity. After the polymorphic RAPD band is cloned and sequenced, the most important task is to design longer, target-specific primers. These new primers bind only to the specific locus of interest, providing much higher reproducibility and potentially allowing for co-dominant scoring, which is more informative.

**169.** (B) Highly genotype-dependent tissue culture requirements.
Doubled Haploid technology is a powerful tool for rapidly creating homozygous mapping populations, but it faces significant biological challenges. The success of the process is often highly dependent on the genotype of the starting material. Many important crop varieties are recalcitrant to the tissue culture and regeneration steps required for DH production, limiting the broad applicability of the technique in some species.

**170.** (A) P-4, Q-3, R-2, S-1
Genetic linkage analysis utilizes various parameters and mathematical models to describe the organization of the genome.
**Mapping Parameters and Definitions:**
P. Recombination Frequency is the proportion of non-parental types.
Q. LOD Score evaluates the statistical likelihood of linkage.
R. Kosambi Function models the presence of crossover interference.
S. Haldane Function assumes crossovers occur completely independently.

**171.** (A) 50 kb/cM
The relationship between physical DNA length and genetic map distance is expressed as the physical-to-genetic ratio. If a 100 kb genomic clone corresponds to a distance of 2 centimorgans, the localized ratio is calculated as 50 kb per cM. This value indicates a high recombination rate in that specific region, as a relatively small physical segment of DNA produces significant genetic exchange.

**172.** (B) Marker band appears only in resistant parent and bulk.
In Bulked Segregant Analysis, a marker linked to a resistance trait will show a distinct pattern of presence and absence between the contrasting pools. If an SSR marker strictly co-segregates with resistance, its characteristic band will be visible in the resistant parent and the resistant bulk. Conversely, the band will be absent in the susceptible parent and the susceptible bulk DNA profile.

**173.** (A) Detection of "ghost QTLs" from multiple linked QTLs.
Composite Interval Mapping is an advanced statistical approach designed to improve the accuracy of QTL localization. It specifically addresses the problem of "ghost QTLs," which are false-positive signals that can appear between two actual, closely linked QTLs. By incorporating other markers as cofactors to account for background genetic variance, CIM can distinguish true loci from these statistical artifacts, providing a more reliable map.

**174.** (C) Pacific Biosciences (PacBio) SMRT Sequencing
Pacific Biosciences SMRT sequencing is a third-generation technology that offers unique advantages for genome assembly. It generates exceptionally long single-molecule reads, often exceeding ten thousand base pairs in length. These long reads are essential for spanning and resolving complex repetitive regions and large structural variations that are difficult for short-read platforms to assemble, leading to much more complete and continuous genomic sequences.

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which ensures that the primer binds at the edge of the repetitive region for consistent amplification.

**192.** (C) Successive selfing accumulates meiotic recombinations.
Recombinant Inbred Line populations provide higher mapping resolution than F2 populations because of the way they are developed. Through several successive generations of self-pollination, the lines accumulate the results of many more meiotic recombination events. These numerous crossovers break the linkage between loci into smaller segments, allowing researchers to pinpoint the location of genes and QTLs with significantly greater precision.

**193.** (B) Linked at specific fraction vs. independent assortment.
The LOD score is a statistical tool used in linkage analysis to determine the physical relationship between loci. It is calculated as the base-10 logarithm of the likelihood ratio comparing two hypotheses. The first hypothesis is that the two loci are linked at a specific recombination fraction, while the second is that they are unlinked and assorting independently according to Mendelian principles.

**194.** (D) Markers are in pericentromeric regions with low crossover.
Large discrepancies between physical and genetic distances often occur due to regional variations in recombination rates. In pericentromeric regions, the DNA is highly condensed into heterochromatin, which significantly suppresses crossing-over. Consequently, a physical distance of several megabases may only correspond to a very small genetic distance, as few recombination events occur to separate markers in those sections of the chromosome.

**195.** (A) Dominant
In Bulked Segregant Analysis, the inheritance pattern of a trait can be inferred from the banding patterns of linked markers. If a marker band from the resistant parent is present in both the resistant and susceptible bulks, it suggests the resistance trait is dominant. This is because both homozygous and heterozygous individuals would be pooled into the resistant bulk, while the susceptible bulk contains only recessive genotypes.

**196.** (C) Exactly one QTL between two flanking markers.
Simple Interval Mapping is a classic statistical framework used to identify Quantitative Trait Loci. The model evaluates the entire chromosomal segment between a pair of adjacent, linked molecular markers. It operates under the specific assumption that exactly one QTL is present within that interval. While powerful, this assumption can lead to errors if multiple linked QTLs are actually present in the same region.

**197.** (A) Spanning repetitive elements with distance constraints.
Repetitive elements in the genome are a major challenge for de novo assembly using short reads. Paired-end and mate-pair sequencing libraries are designed to overcome this by generating pairs of reads from the ends of DNA fragments with a known physical distance. These distance constraints allow bioinformatics algorithms to bridge across repetitive sequences and correctly orient contigs into larger, more continuous scaffolds.

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**198.** (D) Predicting genes based entirely on statistical properties.
Ab initio gene prediction is a bioinformatics approach used to identify functional regions in a genome without relying on comparisons to known genes. These algorithms analyze the DNA sequence for specific statistical properties, such as codon bias, splice site motifs, and promoter signals. By identifying patterns that are characteristic of genes, the software can predict the location and structure of novel genetic features.

**199.** (B) Precise, targeted modification of a predefined sequence.
CRISPR-Cas9 technology has revolutionized functional genomics by providing a tool for precise and targeted genome editing. Unlike chemical mutagens like EMS, which cause random mutations throughout the entire genome, CRISPR-Cas9 can be programmed to modify a specific, predefined DNA sequence. This capability allows researchers to directly investigate the function of a chosen gene by creating precise knockouts or specific sequence alterations.

an individual's genetic merit for a specific trait. A GEBV is calculated by summing the predicted additive genetic effects of thousands of molecular markers distributed across the entire genome. This approach allows breeders to select elite individuals based on their overall genetic potential rather than just phenotypic performance.

**184.** (C) Familial relatedness among panel individuals.
In Genome-Wide Association Studies, the presence of genetic relatedness among individuals in the panel can lead to statistical errors and false-positive associations. To correct for this, researchers include a K-matrix, or kinship matrix, in their mixed linear models. This matrix accounts for the varying degrees of shared ancestry between individuals, ensuring that the identified associations between markers and traits are statistically robust and accurate.

**185.** (D) Multi-locus marker
Molecular markers can be classified based on the number of genomic locations they interrogate in a single assay. A multi-locus marker, such as those generated by RAPD or AFLP, amplifies multiple different DNA segments from across the genome simultaneously. This produces a complex banding pattern that provides a broad overview of genomic variation, making it an efficient tool for diversity analysis and genome scanning.

**186.** (A) PCR inherently provides greater map resolution.
The shift from RFLP to PCR-based marker systems is driven by several practical and technical advantages of PCR. PCR is significantly faster, requires much smaller amounts of DNA, and avoids the use of radioactive probes and Southern blotting. However, map resolution is not inherently higher with PCR; rather, it depends on the number of markers used and the recombination frequency in the population.

**187.** (B) Short decamers require low temperatures to stably bind.
RAPD amplification utilizes short, ten-nucleotide primers that scan the genome for random binding sites. Because these primers are so short, they have low melting temperatures and require relatively low annealing temperatures, typically between 35 and 40 degrees Celsius, to bind stably to the template DNA. These low-stringency conditions are essential for allowing the primers to initiate amplification from multiple genomic locations.

**188.** (A) Optimal fragment sizes and complexity are achieved.
The AFLP technique utilizes a strategic combination of two different restriction endonucleases. A rare cutter, like EcoRI, and a frequent cutter, like MseI, work together to fragment the genomic DNA. This dual-enzyme approach ensures that the resulting DNA fragments are of an optimal size for efficient PCR amplification and subsequent high-resolution separation, while also reducing the overall complexity of the fragment mixture.

**189.** (A) They exhibit hypervariability due to replication slippage.
Simple Sequence Repeats are exceptionally valuable for distinguishing between closely related genotypes due to their high levels of polymorphism. This diversity is driven by the biochemical process of replication slippage, which frequently causes the insertion or deletion of repeat units. The resulting variation in allele lengths at each locus makes SSRs highly informative for mapping, diversity studies, and the identification of individual cultivars.

**190.** (A) I, II, III, IV
The development and application of high-throughput SNP arrays involve several coordinated steps.
**SNP Array Workflow:**
The process begins with whole-genome resequencing to discover variations. Next, bioinformatics is used to select an informative panel of SNPs. These allele-specific oligonucleotides are then synthesized and immobilized onto a microchip. Finally, labeled genomic DNA is hybridized to the chip, and the resulting signals are captured to determine the individual's genotype.

**191.** (D) It uses a specific repeat sequence with short anchors.
While both RAPD and ISSR are PCR-based markers that scan the genome, their primer designs differ fundamentally. RAPD uses arbitrary decamer sequences. In contrast, an ISSR primer is designed based on a simple sequence repeat motif. It also features a short "anchor" of one or two nucleotides at one end,

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chloroplasts are inherited strictly through the maternal lineage, as they are not transmitted through the pollen. This characteristic makes these markers invaluable for studying maternal evolutionary history, seed-mediated gene flow, and the relationships between different maternal lines in diverse germplasm collections.

**209.** (B) Appearance of smears and false-positive extra bands.
Successful RFLP analysis depends on the complete and precise digestion of genomic DNA by restriction endonucleases. If the digestion is incomplete, the enzyme fails to cut at every recognition site, leading to the presence of larger-than-expected DNA fragments. When visualized, this results in the appearance of undesirable smears or false-positive extra bands on the autoradiograph, which can complicate or invalidate the genetic interpretation.

**210.** (A) P-3, Q-4, R-2, S-1
Genomic and proteomic techniques offer diverse ways to characterize biological systems.
**Technique Applications:**
P. RNA-Seq is used for de novo transcript discovery and quantification.
Q. SAGE quantifies transcripts using short concatenated tags.
R. 2D-PAGE provides high-throughput separation of intact proteins.
S. MALDI-TOF is used to identify peptide mass fingerprints for protein identification.

**211.** (A) Implementing a "touch-down" PCR cycling profile.
Reducing non-specific amplification is a key challenge in complex PCR reactions like those in the AFLP protocol. A touch-down PCR profile is a highly effective strategy for improving specificity. By starting with a high annealing temperature and gradually decreasing it, the model ensures that only the most perfectly matched primers bind initially, significantly reducing the background noise and improving the clarity of the results.

**212.** (C) Both the 150 bp and 160 bp bands
SSR markers are co-dominant, meaning they can detect and distinguish both alleles present at a specific locus in a diploid individual. If Parent A and Parent B have different allele sizes, their $F_1$ progeny will inherit one allele from each. When amplified, the $F_1$ genotype will show both characteristic bands on the gel, allowing for the clear identification of heterozygous individuals in the population.

**213.** (A) Exonuclease activity cleaves probe and separates dye.
TaqMan assays achieve highly specific SNP detection through a fluorescence-based signaling system. The dual-labeled probe contains a fluorescent dye and a quencher in close proximity. During the PCR reaction, the 5' to 3' exonuclease activity of the advancing Taq DNA polymerase cleaves the hybridized probe. This cleavage physically separates the dye from the quencher, allowing the fluorescent signal to be emitted and detected.

**214.** (D) High robustness and target-specific amplification.
SCAR markers represent a significant technical improvement over the original RAPD markers from which they are derived. By using longer, target-specific primers designed from the sequenced RAPD fragment, SCAR assays offer much higher robustness and reproducibility. They amplify only a single, specific genomic locus, making them ideal for high-throughput screening in breeding programs where consistent and reliable results are mandatory.

**215.** (B) Doubled Haploids (DH)
Doubled Haploid mapping populations are generated through a combination of specialized techniques. After creating haploid embryos, often through wide crosses or irradiated pollen, the chromosome number is doubled using a chemical agent like colchicine. This rapid process yields plants that are perfectly homozygous at all genetic loci. DH lines are particularly valuable because they provide a genetically fixed and permanent resource for mapping.

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**216.** (B) 5.0 cM apart
In the simplest form of genetic mapping, the recombination fraction between two loci is used as a direct estimate of their map distance. A recombination frequency of 0.05 indicates that recombination occurs between the markers in 5% of the gametes. In the centimorgan system, this 5% frequency is equivalent to a map distance of 5.0 cM, providing a relative measure of their physical proximity.

**200.** (A) Both A and R are true and R is the correct explanation of A.
Microarray technology is a powerful tool for transcriptomics, but it has specific requirements for its design.
The effectiveness of the chip depends on the precise hybridization of sample cDNA to immobilized probes. These probes must be pre-synthesized to match known genetic sequences, meaning that researchers must have prior knowledge of the target genome's sequence to construct a functional microarray for gene expression analysis.

**201.** (B) Matrix-Assisted Laser Desorption/Ionization
Matrix-Assisted Laser Desorption/Ionization is a "soft" ionization technique that is essential for the analysis of large biomolecules in mass spectrometry. By embedding the sample in a specialized matrix, a laser can be used to ionize the proteins without causing them to fragment into small pieces. This allows the mass spectrometer to measure the mass of the intact molecule, which is vital for protein identification.

**202.** (A) LC analyzes non-volatile compounds without derivatization.
Liquid Chromatography offers a distinct advantage over Gas Chromatography in the study of the metabolome. Many biological metabolites are highly polar or non-volatile, making them unsuitable for GC without complex chemical modifications. LC separates these molecules in a liquid mobile phase at moderate temperatures, allowing for the direct analysis of a wider range of compounds without the need for extensive sample derivatization.

**203.** (B) Identifying transporters correlated with accumulation.
Integrating ionomic and transcriptomic data provides a powerful multi-omics approach to understanding plant physiology. While ionomics quantifies the actual accumulation of elements, transcriptomics reveals the activity of the genes involved. By correlating changes in the ionome with the expression levels of specific genes, researchers can identify the particular transporters and regulatory networks that drive the uptake and distribution of minerals under stress.

**204.** (B) Gene duplication events
Homologous genes are categorized based on the evolutionary mechanism that led to their divergence. Paralogues are genes within the same species that have originated from a gene duplication event. Following duplication, these genes may evolve different functions or expression patterns. In contrast, orthologues are genes in different species that diverged from a common ancestor through speciation, typically maintaining the same biological role.

**205.** (A) To fix target genes in a homozygous recessive state.
In a marker-assisted backcrossing program targeting a recessive resistance gene, self-pollination of the final generation is essential. Throughout the backcross generations, the target gene is maintained in a heterozygous state to ensure its transmission. The final self-pollination allows the recessive alleles to segregate, enabling the breeder to select individuals that are homozygous recessive and therefore exhibit the desired resistance phenotype.

**206.** (C) The $p > n$ problem where markers exceed observations
Genomic selection models often face the mathematical challenge of having thousands of markers but only a limited number of phenotypic observations. This is known as the "$p > n$ " problem, which can lead to statistical over-fitting. Ridge Regression BLUP addresses this by applying a shrinkage penalty to the marker effects. This penalty constrains the model, ensuring more reliable predictions of breeding values from the complex genetic data.

**207.** (D) Extremely high density of genome-wide markers.
The mapping resolution of a Genome-Wide Association Study is heavily influenced by the rate of linkage disequilibrium decay in the population. In species where LD decays very rapidly over short physical distances, such as maize, the chance of any single marker being linked to a causative locus is low. To compensate, an extremely high density of markers is required to ensure comprehensive genome coverage.

**208.** (C) Chloroplast specific markers
Chloroplast DNA markers are a specialized tool in genetics because of their uniparental inheritance pattern. In most agronomically important crops,

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**140.** (A) I only
RFLP analysis is a classical molecular marker technique based on DNA restriction patterns. It specifically detects sequence variations that occur within the recognition sites of restriction enzymes. These mutations either create or destroy a site, altering the fragment lengths. Therefore, stating that RFLP inherently detects variations that occur outside of these recognition sites is incorrect, as it is the site change itself that is detected.

**141.** (A) They increase primer dimers and reduce the number of potential genomic binding sites.
In RAPD analysis, the use of short primers is essential for the random scanning of the genome. Extremely long primers are avoided because they have a higher probability of forming primer dimers, which interferes with the reaction. Furthermore, longer primers have much higher specificity, which significantly reduces the number of random genomic locations where they can bind and initiate amplification.

**142.** (C) Rapid genome-wide scanning for polymorphisms.
The AFLP technique is highly valued for its exceptional multiplexing capability, as it can generate dozens of polymorphic bands in a single reaction. This high output makes it an efficient tool for rapidly scanning the entire genome for variations. It is particularly useful for constructing high-density genetic maps and assessing genetic diversity in species where very little sequence information is available.

**143.** (D) Sequencing genomic libraries enriched for repeats
Developing novel SSR markers for a poorly characterized species traditionally involves the creation of genomic libraries that are enriched for repetitive sequences. These libraries are then sequenced to identify the unique DNA flanking regions surrounding the repeats. Once the sequence of these flanking regions is known, specific primers can be designed to amplify the SSR loci for use in genetic studies.

**144.** (C) By allele-specific fluorescent dye emission
KASP is a modern high-throughput genotyping platform that eliminates the need for gel electrophoresis. The system uses two different allele-specific primers, each associated with a unique fluorescent reporter dye. During the PCR reaction, the incorporation of the specific allele results in the emission of its corresponding fluorescent signal. This allows for the rapid and automated detection of SNP genotypes in a microplate format.

**145.** (B) dCAPS introduces a mismatch to create a restriction site.
While both CAPS and dCAPS markers utilize restriction digestion to detect SNPs, they differ in their primer design. CAPS markers target existing restriction sites. In contrast, dCAPS markers are used when an SNP does not naturally create a restriction site. The dCAPS primer is designed with a deliberate mismatch near the SNP, which creates a novel restriction site in only one of the alleles.

**146.** (D) Only the chromosomal segment containing the target.
Near Isogenic Lines are an essential tool in genetic research, developed to study the effect of a single gene in a uniform genetic background. Through several generations of backcrossing, the lines become genetically identical to the recurrent parent for almost the entire genome. They differ only at the small chromosomal segment that contains the specific target gene introgressed from the donor parent.

**147.** (A) They result in underestimation of true genetic distance.
Genetic mapping relies on the frequency of observed recombination events between markers. When markers are far apart, there is a probability that two crossovers will occur in the same interval. These double crossovers essentially restore the parental configuration, making them undetectable in standard analysis. Consequently, failing to account for these hidden events leads to a systematic underestimation of the true genetic distance.

**148.** (C) Cytogenetic mapping (e.g., via Flow Karyotyping)
Cytogenetic mapping involves the direct visualization of genetic sequences on the chromosomes themselves. Techniques such as flow karyotyping can be used to physically isolate individual intact chromosomes based on their size and DNA content. Once isolated, markers or genes can be mapped to specific chromosomal bands or regions, providing a high-level physical

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**217.** (D) Pericentromeric regions with low crossover rates.
The relationship between physical and genetic distances is non-linear and varies across the chromosome. In pericentromeric regions, the DNA is highly condensed, which significantly suppresses meiotic crossing-over. As a result, a very large physical distance in these regions corresponds to only a small genetic distance. On a linkage map, these large segments appear heavily compressed compared to more recombinogenic regions like the telomeres.

**218.** (A) Equal allelic representation across both bulks.
The power of Bulked Segregant Analysis lies in its ability to differentiate linked markers from the background genomic noise. Markers that are randomly dispersed and unlinked to the target trait are expected to segregate independently. Therefore, in the pooled DNA of the two phenotypic bulks, these unlinked markers should show equal representation of alleles from both parents, indicating no association with the trait of interest.

**219.** (B) Composite Interval Mapping (CIM)
Composite Interval Mapping is an advanced algorithmic improvement for QTL localization. It combines the strengths of interval mapping and multiple regression analysis by incorporating markers outside the target interval as cofactors. This approach effectively accounts for the genetic variance of other QTLs elsewhere in the genome, reducing background noise and improving the precision and power to detect and map the target QTL.

**220.** (B) DNA fragmentation → Adapter ligation → PCR → Sequencing
Preparing a DNA library for Next-Generation Sequencing involves a specific sequence of biochemical and molecular steps.
**NGS Library Preparation:**
First, the genomic DNA is fragmented into small, manageable pieces. Next, synthetic adapters are ligated to the ends of these fragments to facilitate binding to the sequencing platform. A PCR step then amplifies the library to ensure sufficient material. Finally, the prepared fragments are sequenced to generate the raw data.

**221.** (A) Paired-end reads with known insert distances.
Assembling continuous genome sequences from short reads is a complex task, especially in repetitive regions. Paired-end reads are essential because they provide information from both ends of DNA fragments with known sizes. These distance constraints allow bioinformatics software to bridge across gaps and repetitive elements, correctly ordering and orienting individual contigs into larger scaffolds, which is vital for high-quality genome reconstruction.

**222.** (C) Forward Genetics
Forward genetics is a classical research approach that moves from an observed phenotype toward the identification of the underlying gene. The process described—inducing mutations, screening the population for a specific abnormal phenotype, and then characterizing the genetic basis of that trait—perfectly defines this methodology. It is a fundamental strategy in biology for discovering the genes that regulate diverse physiological and developmental processes.

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**223.** (D) Short tags may map ambiguously to gene families.
While Serial Analysis of Gene Expression was a groundbreaking tool for transcriptomics, it has a significant limitation compared to modern RNA-Seq. SAGE captures very short sequence tags from the ends of transcripts. These tags are often too short to uniquely identify a specific gene, leading to ambiguous mapping when multiple genes in a family share similar sequences. RNA-Seq provides much longer reads, allowing for more precise identification.

**224.** (C) Identifying in vivo physical protein interactions.
The Yeast Two-Hybrid system is a unique and powerful tool in proteomics for studying the physical interactions between proteins. Unlike many in vitro methods, the Y2H assay detects these interactions in a living cellular environment. By reconstituting a functional transcription factor through the binding of two target proteins, it provides direct evidence of their in vivo association, which is essential for understanding cellular signaling networks.

**225.** (B) Untargeted metabolomics via high-resolution LC-MS
Mapping an entirely unknown metabolic pathway requires a comprehensive and unbiased

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information prevents the application of Hardy-Weinberg statistics and other essential analytical tools for studying genetic structure and diversity within groups.

**234.** (A) To prevent re-cleavage of amplified fragments.
In the AFLP protocol, the restriction enzymes must be inactivated after the initial digestion and ligation steps. This is critical because the subsequent amplification rounds generate many new copies of the DNA fragments. If the endonucleases remained active, they would continue to cut the newly synthesized products at their recognition sites, disrupting the reaction and preventing the formation of the discrete bands required for analysis.

**235.** (B) Location within highly conserved coding sequences.
SSR markers derived from Expressed Sequence Tags offer high transferability across different, related species. This advantage exists because EST-SSRs are located within or very close to the coding regions of genes. These functional sequences are subject to strong evolutionary constraints and are therefore more conserved between species than non-coding genomic regions. This conservation allows the same primers to successfully amplify the target locus in diverse taxa.

**236.** (B) Single-base mismatch discrimination on a surface.
High-throughput SNP genotyping via microarray technology relies on the precise physical interaction between DNA molecules. The microchip contains millions of immobilized probes that are designed to perfectly match specific SNP alleles. The system's effectiveness depends on the ability of these probes to discriminate between sequences that differ by only a single nucleotide mismatch, ensuring that the target DNA only hybridizes to its exact complement.

**237.** (A) Restriction endonucleases
The Cleaved Amplified Polymorphic Sequence marker system is a versatile tool that combines two different molecular techniques. It utilizes the high target specificity and sensitivity of PCR to amplify a specific genomic region. This amplified product is then interrogated by a restriction endonuclease, which identifies single nucleotide variations that affect the enzyme's recognition site. This combination allows for the robust detection of localized sequence polymorphisms.

**238.** (C) Linkage drag
Near Isogenic Lines are developed to study a single introgressed gene in a uniform background. However, it is often difficult to remove all of the donor parent's genetic material near the target locus. This phenomenon is known as linkage drag, where a small chromosomal segment flanking the gene of interest is co-inherited from the donor. This drag can sometimes introduce undesirable traits along with the target gene.

**239.** (D) The Haldane function
The Haldane mapping function is a classic mathematical model used in linkage analysis to convert recombination frequencies into genetic map distances. It uses the specific formula $d = -0.5 \ln(1 - 2\theta)$ to account for the occurrence of multiple crossovers between distant markers. The function operates under the fundamental assumption that crossovers occur independently and randomly along the chromosome, without any interference from one another.

**240.** (C) The mapping resolution or cM/Mb ratio
The relationship between genetic map distance and physical chromosomal length is described by the mapping resolution, often expressed as the cM/Mb ratio. This parameter indicates how much physical DNA sequence corresponds to a specific unit of genetic recombination. Mapping resolution varies significantly across the genome, with high ratios in recombinogenic regions like telomeres and very low ratios in heterochromatic regions like centromeres.

**241.** (B) Simultaneous mapping and massive genome-wide SNP data.
QTL-seq is a modern approach that significantly enhances the power of Bulked Segregant Analysis by integrating it with Next-Generation Sequencing. This methodology allows for the simultaneous mapping of traits and the generation of massive amounts of genome-wide SNP data. By analyzing the SNP-index across the entire genome, researchers can rapidly and precisely pinpoint the genomic regions associated with the target trait in a single experiment.

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analytical approach. Untargeted metabolomics using high-resolution Liquid Chromatography-Mass Spectrometry is the ideal tool for this task. It allows for the simultaneous detection and characterization of thousands of different metabolites in a sample. This global profile enables researchers to identify novel compounds and trace their biochemical relationships to reconstruct complex metabolic pathways.

**226.** (D) An extremely high-temperature argon plasma torch.
Inductively Coupled Plasma Mass Spectrometry relies on a powerful ionization source to analyze the elemental composition of a sample. An extremely high-temperature argon plasma torch is used to generate temperatures reaching up to ten thousand degrees Celsius. This intense heat effectively vaporizes, atomizes, and ionizes the molecules in the sample, allowing for the sensitive and precise detection of trace elements across a broad dynamic range.

**227.** (A) ChIP-seq
Chromatin Immunoprecipitation sequencing is a powerful genomic technique used to map the interactions between proteins and DNA across the entire genome. By cross-linking proteins to DNA and using specific antibodies to isolate the complex, researchers can identify the exact binding sites of transcription factors and other DNA-binding proteins. This provides vital information about the regulatory networks that control gene expression and genome function.

**228.** (C) 75%
In marker-assisted backcrossing, the goal is to recover the genetic background of the recurrent parent while retaining a specific gene from the donor. In the first backcross generation ($BC1F_1$), individuals inherit 50% of their genome from the recurrent parent and 50% from the $F_1$ hybrid. On average, this results in a genome that is theoretically 75% recurrent parent, providing a significant step toward developing a near-isogenic line.

**229.** (A) Only genotypic data to predict breeding values.
Genomic selection significantly accelerates the breeding process by allowing for selection at an early stage. Once a training population has been used to build an accurate prediction model, the model can be applied to a separate breeding population. For these new individuals, only molecular marker data is required to calculate their Genomic Estimated Breeding Values, enabling breeders to select the best genotypes without waiting for phenotypic evaluation.

**230.** (A) I, II, III, IV
Executing a robust Genome-Wide Association Study requires a logical and chronological series of research steps.
**GWAS Operational Sequence:**
First, a diverse panel of individuals is assembled and their phenotypes are recorded. Next, the entire panel is genotyped with high-density markers. Statistical models are then applied to the data, incorporating corrections for population structure and kinship. Finally, the significant associations between markers and traits are identified based on established p-value thresholds.

**231.** (B) Geometric amplification of targets by polymerase.
The exceptional sensitivity of PCR-based marker systems is due to the fundamental biochemical mechanism of the polymerase chain reaction. During the cycling process, the target DNA sequence is doubled in each round, leading to geometric amplification. This allows millions of copies of the specific locus to be generated from a very small starting amount of template DNA, making these markers ideal for high-throughput genetic analysis.

**232.** (C) One
An RFLP probe is designed to be complementary to a specific genomic sequence. If the probe hybridizes to a single-copy gene in a homozygous diploid individual, it will find the same target sequence on both homologous chromosomes. After restriction digestion, these two identical segments will produce the same fragment size. Consequently, only a single, discrete band will be visible on the Southern blot for that individual.

**233.** (D) Allele frequencies and Hardy-Weinberg statistics.
The dominant nature of RAPD markers presents a major limitation for population genetics. Because the technique cannot distinguish between homozygous dominant and heterozygous genotypes, researchers cannot accurately determine the frequency of the individual alleles in the population. This lack of detailed genotypic

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This structural similarity allows researchers to utilize well-characterized model genomes to predict the location and function of genes in more complex genomes. Such comparative approaches significantly accelerate gene discovery and the development of molecular markers across diverse taxa.

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**242.** (D) Highest QTL probability is located between markers.
Interval Mapping is a powerful statistical framework for QTL localization that analyzes the entire chromosomal segment between a pair of linked markers. When the likelihood ratio peaks at a specific position within the interval, it indicates that the highest probability of the QTL being present is at that exact location. This approach provides a much more precise and informative estimate of QTL position than single-marker analysis.

**243.** (A) Subdivide complex genomes into ordered fragments.
Hierarchical Clone-by-Clone sequencing was developed to manage the immense complexity of large eukaryotic genomes. The strategy involves creating an initial physical map by organizing large DNA inserts from BAC libraries into an ordered set of overlapping clones. This structured approach allowed researchers to subdivide the genome into smaller, manageable fragments that could be sequenced and then precisely reassembled into a complete and continuous genome sequence.

**244.** (C) Pseudogenes
Pseudogenes are genomic sequences that share significant homology with functional genes but have lost their ability to code for proteins. This loss of function is typically due to the accumulation of debilitating mutations, such as premature stop codons or frameshifts, within the coding sequence. Identifying and annotating pseudogenes is an important task in genomics for understanding the evolutionary history of gene families and the processes of genome decay.

**245.** (A) Sequencing flanking DNA using transposon primers.
In a transposon-tagging experiment, the identification of a mutant phenotype provides a direct link to the causative gene. Because the transposon sequence is known, it serves as a molecular anchor. The most direct way to identify the disrupted gene is to use primers specific to the transposon to sequence the adjacent genomic DNA. This flanking sequence data can then be used to locate and characterize the gene.

**246.** (D) The 3' poly-A tail
Eukaryotic messenger RNA possesses a unique structural feature that is widely exploited in transcriptomic research. Most mature mRNAs have a long string of adenine nucleotides at their 3' end, known as the poly-A tail. By using beads coated with complementary oligo-dT sequences, researchers can selectively capture mRNA from a complex biological sample, effectively removing the overwhelming amount of ribosomal RNA that does not have this feature.

**247.** (B) To generate predictable peptides for better mass spectrometry
Trypsin is the most commonly used protease in "bottom-up" proteomics due to its highly specific and predictable cleavage patterns. It cuts proteins exclusively at the carboxyl side of lysine and arginine residues. This generates a set of peptides that are of a suitable size and charge for efficient separation and ionization in mass spectrometry, facilitating more accurate and reliable protein identification from complex mixtures.

**248.** (C) Local electronic and chemical environments.
In Nuclear Magnetic Resonance spectroscopy, the chemical shift of an atom's nucleus is a highly sensitive measure of its local environment. The exact frequency at which a nucleus resonates is influenced by the surrounding electrons and nearby chemical bonds. This information provides detailed insights into the molecular structure and functional groups of metabolites, allowing for their unambiguous identification and structural elucidation in complex biological samples.

**249.** (D) Elemental crosstalk or interaction networks
Ionomics explores the complex networks of mineral homeostasis, where the accumulation of one element is often linked to the levels of others. This concept of elemental crosstalk is essential for interpreting data where a deficiency in one nutrient, like zinc, triggers the hyperaccumulation of another, such as iron. These interactions reflect the shared transporters and regulatory pathways that plants use to manage mineral uptake and balance.

**250.** (A) Predict gene locations based on model genomes.
The concept of collinearity describes the conservation of gene order along chromosomal segments among related species, such as the major cereal crops.

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